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NUTRIENT AND TOXIC EFFECTS OF CERTAIN IONS ON CITRUS AND WALNUT TREES WITH ESPECIAL REFERENCE TO THE CONCENTRATION AND P_H OF THE MEDIUM

BY

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CONTENTS

PAGE

INTRODUCTION 1

EXPERIMENTAL DATA AND DISCUSSION 2

I. The effect of sodium sulfate on walnut seedlings (*Juglans regia*) 2

II. The effect of sodium nitrate on walnut seedlings 6

III. The absorption of sodium and chlorin from nutrient solutions of different concentration 12

IV. The chlorin content of plants grown in solutions of different chlorids 13

A. Walnut seedlings 13

B. Citrus seedlings 16

V. The effect of different salts of sodium 18

VI. The effect of alkalinity on growth and sap reaction 21

A. Walnut seedlings 21

B. Wheat seedlings 22

VII. The relation of H-ion concentration to the absorption of chlorids 23

A. Citrus seedlings 23

B. Walnut seedlings 30

VIII. The effects of sodium chlorid on orange trees in soil, and the results of leaching 32

A. Trees under controlled conditions 33

1. Sodium chlorid added to non-saline soil 33

a. Changes in the soil 33

b. Effects on the trees 35

c. The chlorin content of the leaves 40

2. Soil previously made saline by orchard irrigation 41

B. Trees grown in the field 45

IX. The effect of sodium bicarbonate on young orange trees 48

X. Relations between calcium deficiency and chlorosis 59

XI. Interrelations between potassium and calcium 61

XII. Effects of magnesium deficiency on young orange trees 69

SUMMARY 70

LITERATURE CITED 73

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INTRODUCTION

The continuation of studies which have been presented in publications from this laboratory[17, 18, 19] has led to the results presented in this paper which should be regarded as a report of work in progress.

The absorption of ions and their distribution in the plant are not determined by such relatively simple factors as the concentration or the solubility of the substance in question, but are profoundly influenced by the coördinating activities of the living plant. An ion may have a specific effect upon a single organ and have little or no direct effect upon others. It is therefore important to study the activity of the plant in conjunction with its chemical analysis, if we are to appreciate the equilibrium existing between the plant and its surroundings. The farmer is obviously interested in maintaining an equilibrium suited to the proper physiological functioning of the plant. The nutrient or toxic effects of certain of the compounds to be discussed are problems of agriculture in semi-arid regions where irrigation is practiced. The present studies are intended to extend our knowledge of the effect of certain factors upon the growth and composition of

* Paper No. 110, University of California, Graduate School of Tropical Agriculture and Citrus Experiment Station, Riverside, California.

young citrus and walnut trees. The present conditions of experimentation in this field of investigation are such that it is necessary to employ young seedling plants for some of the work. It is hoped that the investigation may be extended to include experiments on the absorption process in older trees.

EXPERIMENTAL DATA AND DISCUSSION

I. The Effect of Sodium Sulfate on Walnut Seedlings

(*Juglans regia*)

The following experiments were designed to show the effects of sodium sulfate added to a well-balanced nutrient solution (Hoagland's). The seedlings were grown two months in 40-liter water cultures in the glasshouse. Twelve uniform walnut seedlings whose radicles were from 6 to 10 cm. long were suspended in each culture through holes in paraffined wooden covers which supported the young plants. Two jars were used for each concentration of the culture solution. The solutions were changed at the end of the first month, more frequent changes being considered unnecessary since the concentrations were high and the volume of the solution per plant large. The volume of the solutions was maintained by frequent additions of distilled water. The illustrations show that walnut seedlings may be successfully grown in a nutrient solution by this means.

The seedlings of series A (table 1) grew in Hoagland's nutrient solution, which has the following composition expressed as parts per million:

Na	K	Ca	Mg	Fe	Mn	NO_3	Cl	SO_4	PO_4	Total
7	185	159	54	1	0.1	718	10	216	105	1455.1

Series B, C, D, and E contained this solution plus 1500, 3000, 4500, and 6000 p.p.m. sodium sulfate, respectively. Since the highest total concentration was approximately 7500 p.p.m., we employed another series (series F) which contained the same salts as series A, but in five-fold concentration.

The seedlings made very satisfactory growth in series A, B, and F (fig. 1). In the higher concentrations the growth of tops and roots was restricted, and the leaf margins were dead like those shown in

TABLE 1

GROWTH AND COMPOSITION OF WALNUT SEEDLINGS IN NUTRIENT SOLUTIONS CONTAINING VARIOUS CONCENTRATIONS OF SODIUM SULFATE

Series	A		B		C		D		E		F	
Parts per million Na in culture solution	7		507		1007		1507		2007		35	
Parts per million SO_4 in culture solution	216		1259		2302		3345		4388		1080	
Parts per million total solutes in culture solutions	1455		3000		4541		6084		7627		7275	
Fresh weight per 10 plants (grams)	Tops 114	Roots 96	Tops 97	Roots 100	Tops 55	Roots 80	Tops 26	Roots 63	Tops 22	Roots 45	Tops 93	Roots 110
Constituents of the dry matter (per cent of ash)	6.73	3.43	6.71	4.85	8.42	5.92	8.34	4.87	7.13	4.74	10.55	10.44
Non-volatile SO_4	.10	.11	.35	.58	.75	.95	.90	.82	.78	.81	.56	.48
Total sulfur (expressed as SO_4)	.45	.47	.68	.79	1.07	1.14	1.29	1.33	1.17	1.54	.79	.93
Na	6.95	8.47	10.29	24.31	11.93	26.02	19.97	26.11	22.68	25.25	8.98	5.13
K	29.37	17.69	32.47	12.53	26.81	11.22	24.21	11.34	25.35	10.84	29.92	19.07
Ca	6.78	4.65	5.69	2.33	3.31	.67	2.24	1.56	1.54	1.38	6.94	3.93
Mg	3.60	1.75	3.80	1.17	2.85	.81	2.86	1.46	2.39	1.31	3.79	1.27
Cl	1.12	.93	1.23	2.09	.94	1.88	1.13	1.54	1.08	1.45	.94	1.01
SO_4	1.96	3.24	5.16	12.04	8.83	16.07	10.78	16.73	10.96	17.14	5.29	4.63
PO_4	16.24	51.77	17.88	39.21	17.61	34.61	20.64	41.00	17.71	41.65	17.39	32.51

fig. 2. The roots were less profusely branched and were dark colored. Growth of the lateral rootlets soon stopped and their apical portions were swollen like those shown in fig. 3. The seedlings of series F showed no abnormality although the initial concentration was 7275 p.p.m.; they differed from those grown in series A chiefly in the smaller growth of epicotyls and leaves.

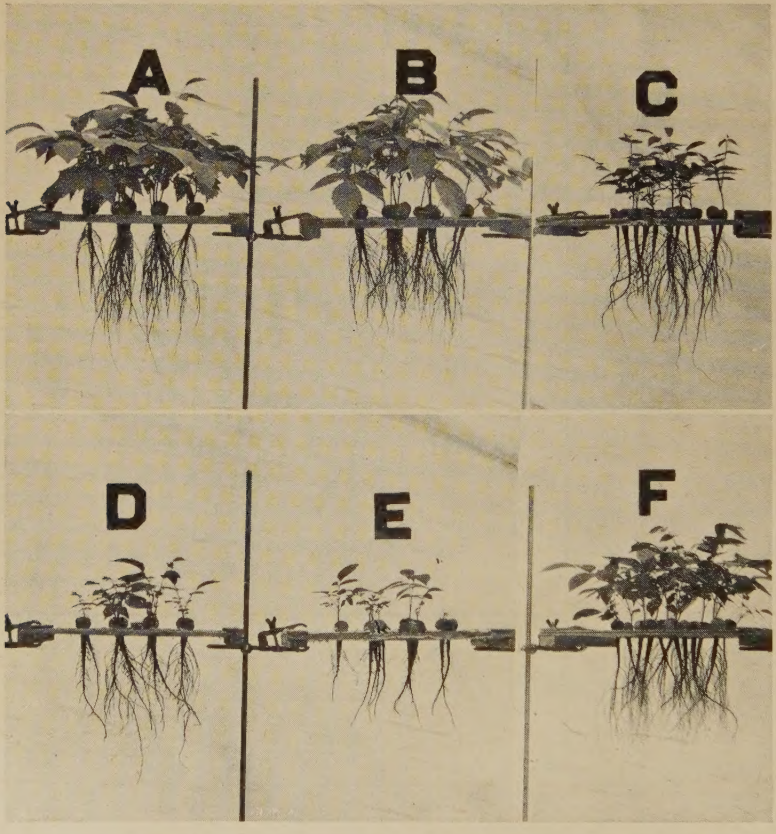

Fig. 1. Walnut seedlings which had grown two months in nutrient solutions containing sodium sulfate. A, in single-strength nutrient solution; B, C, D, E, in single-strength nutrient solution plus 1500, 3000, 4500, and 6000 p.p.m. sodium sulfate respectively; F, in nutrient solution having five times the ordinary concentration.

The fresh weight of the tops and roots of the plants (without cotyledons) in each series is given in table 1. It will be noted that growth of tops was more retarded by the higher concentrations of Na_2SO_4 than that of roots, in fact the epicotyls entirely failed to

develop in some plants in the higher concentrations. The maximum ash content was found in the plants grown in series F.

The sulfur content of the plants, whether measured by the non-volatile SO_4 or as total sulfur in the dry matter, reflected in a general way the amount of sulfate in the culture solution. There appears to

Fig. 2. Walnut leaf from plant grown in culture solution containing 4500 p.p.m. sodium sulfate. The dead margins were characteristic of the plants grown in the higher concentrations of sodium sulfate.

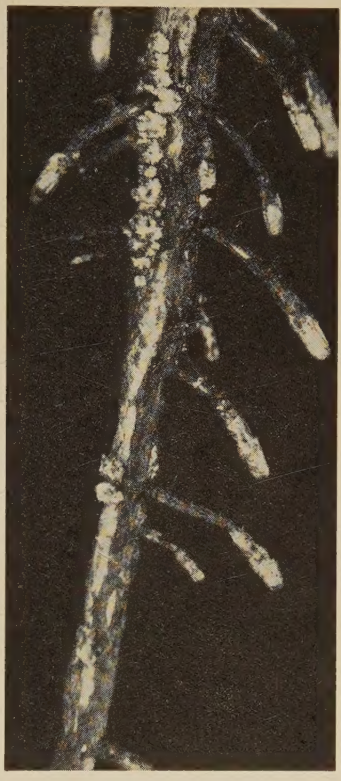

Fig. 3. Walnut root from culture solution containing more than 4000 p.p.m. sodium sulfate.

have been an increase in both the inorganic and organic compounds of sulfur.

The analyses of the ash show that active absorption of certain ions took place. The percentages of Na and of SO_4 reflected the influence of these ions in the culture solutions up to a certain limit, beyond which an increase in the concentration had no effect.

The ash of the roots contained a higher per cent of Na than the tops with the exception of plants in series F. Where the culture solution contained 500 or more p.p.m. Na the per cent of this ion found in the ash of roots was nearly constant.

The ash of the tops contained a higher percentage of K than that of the roots. Although the percentage of K in the tops was fairly uniform in all series, the percentage of K in the roots was lower when the concentrations of Na_2SO_4 in the solutions were higher.

The percentages of Ca and Mg although small, show a rather striking relation to the amounts of Na_2SO_4 in the culture solution. The higher concentrations of Na_2SO_4 seem to have diminished the absorption of Ca and Mg.

The roots were richer in SO_4 than the tops, except in series F. Series C, D, and E were rather uniform in size (cf. fig. 1) and contained considerably higher amounts of SO_4 (series A, B, and F). With the data at hand it is not possible to relate the growth entirely to the amounts of Na or of SO_4 found in the plants. We are inclined to believe that the retarded growth in series C, D, and E is correlated to some extent with the lower percentages of Ca absorbed by the plants. In view of the conspicuous changes in the size and shape of lateral roots of the injured plants it is not improbable that their ability to absorb ions was modified because of altered arrangement and structure of the cells. The microscopical structure of the malformed roots awaits study.

II. The Effect of Sodium Nitrate on Walnut Seedlings

Walnut seedlings were grown from April 11 to June 5 as water cultures in 2-quart glass jars, with one seedling in each jar. Each series contained 12 jars. The initial concentration of Na and NO_3 is given in table 2. The solutions were renewed every two or three weeks.

With solution G (table 2) as a basis the other solutions were made by adding 1324, 2647, 3971, and 5295 parts per million of $NaNO_3$, respectively. The total concentrations are given near the top of table 2.

The growth of the walnut seedlings was similar to that in the preceding experiment, although the roots were less affected by the higher concentrations of $NaNO_3$. The greatest effect was noted in

TABLE 2

GROWTH AND COMPOSITION OF WALNUT SEEDLINGS IN NUTRIENT SOLUTIONS CONTAINING VARIOUS CONCENTRATIONS OF SODIUM NITRATE

Series	G		H		I		J		K	
Parts per million Na in culture solution	7		366		724		1083		1442	
Parts per million NO_3 in culture solution	718		1683		2648		3613		4578	
Parts per million total solutes in culture solution	1455		2780		4102		5426		6750	
Fresh weight per 10 plants (grams)	Tops 77	Roots 92	Tops 72	Roots 90	Tops 46	Roots 75	Tops 36	Roots 71	Tops 31	Roots 90
Constituents of the dry matter (per cent):										
Ash	4.88	3.46	5.25	3.95	5.64	4.16	5.98	4.82	7.32	5.28
Total N	2.68	3.31	3.16	3.63	3.54	4.17	3.80	4.66	3.76	4.73
Constituents of the ash (per cent):										
Na	6.49	7.92	12.88	15.76	16.53	16.47	18.78	22.65	21.01	21.43
K	26.78	25.86	27.68	19.05	26.80	15.75	21.07	18.07	19.18	17.53
Ca	8.09	2.92	6.17	1.45	3.21	0.99	3.32	0.78	2.39	1.18
Mg	4.20	2.38	3.67	1.08	2.56	1.18	2.60	1.18	1.84	1.56
SO_4	2.57	3.74	2.98	5.26	2.88	7.08	3.90	8.75	3.10	8.18
PO_4	19.92	54.95	18.78	48.90	19.66	50.68	21.40	46.17	18.27	42.71

the tops of the seedlings. The leaves of plants in series I, J, and K showed dead margins somewhat as illustrated in fig. 2. The injury was increasingly severe in the higher concentrations of $NaNO_3$. Since the seedlings in solution F (table 1) showed no dead leaf margins when the total concentration of salts was 7275 p.p.m., it is improbable that the injury was caused solely by concentration. The root systems were well developed and healthy in appearance. The size of the plants is indicated by the fresh weights. It is interesting to note that the growth in series H was as good as in G, although the former solution contained over 1600 p.p.m. NO_3. The inhibiting effects of larger concentrations of $NaNO_3$ appear to be shown mainly by the epicotyls.

The percentages of ash and of nitrogen were increased by the higher concentrations of $NaNO_3$ although not proportionally to the concentration.

The percentage of Na in the tops and roots increased up to a certain point, beyond which no significant increase was found. The percentages of Ca and of Mg were substantially reduced by the more concentrated solutions.

In another experiment, walnut seedlings were grown two months in Hoagland's nutrient solution which contained $NaNO_3$ or Na_2SO_4 in concentrations of $\frac{M}{46}$. The solutions were renewed every two or three weeks. Duplicate cultures were made in stone jars holding 40 liters with 13 seedlings in each jar.

Both series made fairly good growth, though the leaves in the Na_2SO_4 series showed some dead margins. The roots in both sets developed normally. The general appearance of the seedlings at the end of two months is shown in fig. 4. The fresh weight of 10 plants from the $NaNO_3$ series was 194 g. and that of 10 plants from the Na_2SO_4 series was 132 g.

The interpretation of this experiment is complicated by the fact that to one solution twice as much Na was added as to the other, but with these equi-molecular solutions the growth was better in the $NaNO_3$ set.

The influence of the cation on walnut seedlings was further studied by an experiment consisting of three series of cultures. The first contained Hoagland's nutrient solution in four times the usual concentration; the second, Hoagland's nutrient solution (ordinary strength) plus 2692 p.p.m. NO_3 as $Ca(NO_3)_2$; the third, Hoagland's nutrient

solution (ordinary strength) plus 2692 p.p.m. NO_3 as $NaNO_3$. The seedlings were grown for six weeks and the fresh weights of 10 plants were 150 g., 152 g., and 84 g., respectively.

These results indicate that, in these high concentrations Na was much less favorable to growth than Ca, and that SO_4 is more toxic than equivalent concentrations of NO_3.

Fig. 4. Walnut seedlings grown two months in nutrient solutions plus equi-molecular concentrations of sodium salts.
N $\frac{M}{46}$ sodium nitrate; S, $\frac{M}{46}$ sodium sulfate.

TABLE 3

EFFECT OF DIFFERENT CONCENTRATIONS OF CULTURE SOLUTION CONTAINING 500 PARTS PER MILLION NaCl, UPON GROWTH AND COMPOSITION OF ORANGE SEEDLINGS

Series	L		M		N		O	
	Culture solution		Culture solution plus 500 p.p.m. NaCl		Double-strength culture solution plus 500 p.p.m. NaCl		Triple-strength culture solution plus 500 p.p.m. NaCl	
Total concentration of solutes (p.p.m.)	1455		1954		3408		4862	
	Tops	Roots	Tops	Roots	Tops	Roots	Tops	Roots
Fresh weights of 100 plants (grams)	227	160	169	113	236	136	192	108
Dry weights of 100 plants (grams)	72	33	50	25	66	29	57	25
Per cent of ash in the dry matter	10.40		11.94		12.39		13.32	
Na (per cent of ash)	5.22		13.13		11.57		9.78	
Cl (per cent of ash)	.75		9.52		7.94		6.86	

TABLE 4

COMPARATIVE EFFECTS OF NaCl AND $CaCl_2$ UPON GROWTH AND ABSORPTION BY WALNUT SEEDLINGS

Series	P		Q		R		S		T	
P.p.m. Cl added to the culture solution	1542		1542		1542		1542			
P.p.m. Na added to the culture solution	1000		0		1000		0			
P.p.m. Ca added to the culture solution	0		871		0		871			
Concentration of nutrient solution (p.p.m.)	1455		1455		7275		7275		5820	
Total concentration of solutes (p.p.m.)	3997		3868		9817		9688		5820	
Fresh weights per 10 plants (grams)	Tops 27	Roots 57	Tops 37	Roots 61	Tops 16	Roots 38	Tops 19	Roots 40	Tops 82	Roots 68
Per cent of ash in the dry matter	7.66	4.80	9.51	5.50	9.62	6.54	9.27	7.32	9.66	8.10
Constituents of the ash (per cent):										
Na	15.16	18.94	5.70	7.21	13.73	9.14	5.76	3.28	8.19	6.48
K	26.68	17.05	25.20	22.88	30.83	20.05	26.30	17.93	30.83	19.59
Ca	4.66	1.20	12.64	4.96	3.60	2.70	7.39	5.77	6.51	9.04
Mg	3.09	1.42	2.34	2.13	2.59	3.10	2.55	2.52	3.61	1.75
Cl	10.17	9.23	12.87	10.10	9.30	6.01	9.29	3.86	1.24	1.60
SO_4	3.41	2.91	2.33	3.48	5.31	4.13	5.13	5.40	4.34	6.82
PO_4	23.13	47.52	15.45	39.82	21.01	50.34	20.81	49.00	2.65	5.10

III. The Absorption of Sodium and Chlorin from Nutrient Solutions of Different Concentration

It has been known for a long time that within a certain range there is some sort of relation between the concentration of ions in a solution and the amount absorbed. The data given above confirm the earlier results on this particular point. We wish to present some data which show that the absorption of an ion is affected by the concentration of other ions. In this instance 500 parts per million NaCl were added respectively to several concentrations of standard nutrient solution.

The experiments were made with St. Michael orange seedlings. Series L (table 3) contained Hoagland's nutrient solution during both periods of the experiment. Series M, N, and O contained Hoagland's solution plus 500 p.p.m. NaCl for 158 days (August 14 to January 19) and then the cultures were grown 33 days in solutions having increased concentrations of nutrient solution plus 500 p.p.m. NaCl. When the experiment was terminated no serious toxic effects were observed in any of the plants, although there were appreciable differences in the sizes of the plants.

Growth was most retarded in series M where the NaCl was added to single-strength nutrient solution. In series N the growth was practically as good as in series L which had no NaCl, which may indicate that the higher concentration of nutrient ions decreased the toxicity of the NaCl. In series O the plants were about the same as in series M, this fact possibly indicating that the concentration of solutes had in itself an inhibiting influence on growth. The percentage of ash in the dry matter progressively increased with increasing concentration of the solution. The percentages of Na and of Cl in the ash were greatest in series M with a progressive decrease in N and O. It would appear that there was something like a dilution of the NaCl due to the higher concentration of nutrient ions.

We may conclude therefore that (within certain limits of concentration) it is not alone the absolute amount of an ion that determines the amount that will be absorbed, but that its relation to the concentration of the other ions is also of considerable importance.

IV. The Chlorin Content of Plants Grown in Solutions of Different Chlorids

a. Walnut seedlings.

The question of the influence of cation upon the absorption of anion has been frequently raised in these and other experiments. Plate[16] observed that the growth of oat seedlings in gram-molecular solutions of chlorids of the different monovalent cations was by no means equal. The growth of tops in the various chlorids formed a descending series as follows: $NH_4 > K > Na > Rb > Li > Cs$. The growth of roots formed a similar series except that the positions of K and Na were reversed. Hoagland[4] found that there were no significant differences in the amounts of Cl absorbed from different salt solutions.

For the next experiment a concentration of 1542 p.p.m. Cl was used in conjunction with the standard nutrient solution (series P and Q, table 4) and with a five-fold concentration of the nutrient solution (series R and S). The control (series T) consisted of a four-fold concentration of nutrient solution. The figures at the top of table 4 show the initial amounts of the ions and the total concentration of the culture solutions. Four series of 26 walnut seedlings each were grown in 40-liter jars, from September 19 to October 30, the solutions being renewed every two to three weeks.

The seedlings in the first four series showed the toxic effects of the chlorids (fig. 5). The plants in series P (NaCl) were characterized by small leaves and a restricted growth of lateral roots, while those in cultures to which $CaCl_2$ was added (series Q) produced good leaves and tops. The primary roots in the NaCl cultures were brown, while those in the $CaCl_2$ cultures were white and thickened. The lateral roots in both series showed restricted growth (fig. 5), and those in series P (NaCl) were short and brown. The roots grew well in series Q ($CaCl_2$) for a time, but decay set in at the upper end of the laterals near the primary root and eventually extended down to the tips. Growth in the more concentrated solutions (R and S) was more limited than in the other two series. The plants in the R series (NaCl) produced short epicotyls and small leaves. In the S series ($CaCl_2$) the epicotyls were longer and the leaves were somewhat larger, but many leaves showed the type of "burning" which is characteristic of high salt concentrations.

Certain relations between absorption and composition of culture solution are shown in table 4. Attention is directed to the fairly constant ratio of chlorin to ash constituents in the four series. Where growth was approximately equal, there was practically the same absorption of Cl without regard to the amount of Na or Ca present. Plants in the concentrated solutions absorbed somewhat less Cl than those in the dilute solutions, probably as a result of the introduction of additional ions.

Fig. 5. Comparative effects of sodium chlorid and calcium chlorid on the growth of walnut seedlings. P, 1542 p.p.m. Cl as sodium chlorid plus single-strength nutrient solution; Q, 1542 p.p.m. Cl as calcium chlorid plus single-strength nutrient solution; R, 1542 p.p.m. Cl as sodium chlorid plus concentrated nutrient solution; S, 1542 p.p.m. Cl as calcium chlorid plus concentrated nutrient solution (cf. table 4).

The results of these series of cultures show that the toxic effects of NaCl and $CaCl_2$ on walnut seedlings, while similar, are not identical. In series P and Q, where the total concentration was not detrimental, NaCl appeared to check the growth of the tops more than $CaCl_2$. In series R and S where the total concentrations were high, there was less difference in the effects of the two salts.

Another experiment with walnut seedlings was made to compare the effects of NaCl and KCl. Three series of cultures were made in 40-liter jars. The first received the nutrient solution in three times the ordinary concentration; the second and third received 1542 p.p.m.

Cl as KCl and NaCl respectively (table 5). The cultures were started December 27 and ended February 7 and the solutions were renewed every two or three weeks.

The growth of the seedlings in the NaCl series was somewhat better than that made in cultures conducted during the summer or early fall. The tops in the NaCl series showed no tendency towards burning like that formerly observed and the growth of roots was not so greatly retarded, although the development of lateral roots was conspicuously inferior to that of the control series (fig. 6).

Fig. 6. Comparative effects of sodium chlorid and of potassium chlorid on the growth of walnut seedlings. U, nutrient solution having three times the ordinary concentration; V, 1542 p.p.m. Cl as potassium chlorid plus nutrient solution; W, 1542 p.p.m. Cl as sodium chlorid plus nutrient solution.

TABLE 5

GROWTH OF WALNUT SEEDLINGS IN NUTRIENT SOLUTIONS CONTAINING EQUIVALENT CONCENTRATIONS OF Cl FURNISHED AS NaCl OR AS KCl.

Series	U		V		W	
P.p.m. Cl added to the culture solution			1542		1542	
P.p.m. Na added to the culture solution					1000	
P.p.m. K added to the culture solution			1700			
Concentration of nutrient solution (p.p.m.)	4365		1455		1455	
Total concentration of solutes (p.p.m.)	4365		4697		3997	
	Tops	Roots	Tops	Roots	Tops	Roots
Fresh weight per 10 plants (grams)	52	110	81	104	40	90

The leaves in the KCl series, although large and broad, were pale green and eventually became yellow. The roots, though well branched, stopped growing and began to die before the end of the experiment. The injury to the lateral roots, like that previously noted in toxic concentrations of $CaCl_2$, was first noticed in the region next to the primary root, from which it extended toward the apex. The plants in the control series were healthy during the entire period of the experiment and produced splendid root systems.

The quantitative relations of the three series are shown by the fresh weights of the plants given in table 5. The weight of roots showed no significant differences, but the weights of tops were quite different. The figures indicate a distinct toxic action of the NaCl and a pronounced increase in growth in the KCl series. The increase in the latter was made during the first part of the experimental period; subsequently, the toxic effects gradually appeared. The condition is somewhat similar to that seen when equivalent amounts of $CaCl_2$ were employed in former experiments.

There is an obvious difference in this case between the toxic effects of NaCl and KCl when equivalent amounts of Cl are used. It might be concluded that an early toxic effect of Cl was more or less prevented by the K ions. It is obvious that there is a time factor involved in the case of toxicity. The toxic effect of $CaCl_2$ appears later than that of NaCl, but it may eventually terminate growth of the plant.

b. Citrus seedlings.

A series of cultures of rough-lemon seedlings was grown for 32 days in a complete nutrient solution with the addition of sodium or calcium chlorid. Eighteen jars each containing three seedlings were used for each concentration. The controls were grown in nutrient solution; the second set was grown in nutrient solution plus 308, 617, 1234 or 1851 p.p.m. chlorin, as sodium chlorid; the third set of plants was grown in solutions the same as the second except that the chlorin was added as calcium chlorid.

The seedlings grown in solutions containing 308 p.p.m. sodium chlorid showed no injury, either to roots or tops; those in solutions containing 617 p.p.m. showed a small amount of injury to the older leaves. The symptoms of injury were a recurved condition and death of the tissues at the margin of the leaf. When the concentration was greater than 617 p.p.m. chlorin, the amount of injury was greater. There was an appreciable, although small, stimulation to growth in all

TABLE 6

GROWTH AND COMPOSITION OF WALNUT SEEDLINGS IN RELATION TO EQUIVALENT AMOUNTS OF VARIOUS ANIONS FURNISHED WITH SODIUM

Series	AA		BB		CC		DD		EE	
P.p.m. Na in culture solution	1000		1000		1000		1000		28	
P.p.m. of anion added	2692 NO_3		1542 Cl		2649 HCO_3		2086 SO_4			
Concentration of nutrient solution (p.p.m.)	1455		1455		1455		1455		5820	
Total concentration of solutes (p.p.m.)	5147		3997		5104		4541		5820	
	Tops	Roots	Tops	Roots	Tops	Roots	Tops	Roots	Tops	Roots
Fresh weight per 10 plants (grams)	44	40	41	63	20	54	42	52	82	68
Per cent of ash in the dry matter	8.26	6.24	8.28	5.96	6.59	4.88	7.66	6.30	9.66	8.10
Constituents of the ash (per cent):										
Na	17.25	23.10	17.38	21.38	25.34	24.54	15.24	25.41	8.19	6.48
K	24.41	12.34	26.90	11.86	28.18	11.39	29.28	7.73	30.83	19.59
Ca	5.57	2.83	3.34	1.87	1.46	2.61	3.83	1.72	6.51	9.04
Mg	3.40	1.24	2.32	.97	2.07	1.35	2.91	.91	3.61	1.75
Cl	1.04	2.29	13.22	14.48	.92	1.84	.98	1.86	1.24	1.60
SO_4	2.74	9.18	3.06	3.91	4.32	9.73	9.52	16.89	4.34	6.82
PO_4	8.83	11.55	9.30	12.71	21.16	11.15	5.73	8.15	2.65	5.10

of the jars which contained 2000 p.p.m. sodium chlorid. Equivalent concentrations of chlorin as calcium chlorid were somewhat less toxic to the plants. The nutrient solution employed contained 159 p.p.m. calcium, yet the addition of calcium chlorid appears to have had a distinctly beneficial effect upon the growth of the plants during the short period of the experiment. Valencia orange trees grown for a longer time in sand cultures receiving 1770 p.p.m. chlorin as calcium chlorid, have shown severe tip burn of the leaves with their subsequent abscission.

We have previously shown[18] that orange trees made poor growth in sand cultures when furnished 1000 p.p.m. NaCl, and that growth was much better when also 440 p.p.m. $CaCl_2$ were added. The difference should be referred to the fact that the first lot suffered, not only from the toxic action of NaCl, but also from the lack of Ca. The introduction of $CaCl_2$ improved conditions for growth in spite of the fact that it increased the concentration of Cl ions. So long as the concentration of Cl is not too high, the presence of Ca may tend to offset the injurious effect of Cl by promoting growth and hence diluting the concentration of Cl in the plant, but in higher concentrations of Cl, or with longer time, injury ultimately appears.

V. The Effect of Different Salts of Sodium

We have discussed the effect of equal concentrations of Cl as NaCl and as $CaCl_2$, and shall now consider the toxic effects of equal concentrations of Na when added in the form of $NaNO_3$, NaCl, $NaHCO_3$, or Na_2SO_4. Five series of walnut seedling cultures were prepared according to the plan shown at the top of table 6. Culture EE contained Hoagland's nutrient solution of four times the usual concentration and may be regarded as the control. Each series contained 26 walnut seedlings, which were grown from July 17 to September 1. It will be seen that each of the four toxic solutions contained 1000 p.p.m. Na. Previous experiments had shown that this concentration of Na salts is decidedly toxic to walnut seedlings. The control solution had only 28 p.p.m. of Na, but its total concentration of solutes was 5800 p.p.m. at the outset. In spite of this high concentration there was no evidence of harmful effect.

The general appearance of the plants from the different solutions at the end of the experiment is shown in fig. 7, and the fresh weights of tops and roots are shown by diagram 1.

Fig. 7. Walnut seedlings showing effects of 1000 p.p.m. sodium in combination with different anions. AA, sodium nitrate; BB, sodium chlorid; CC, sodium bicarbonate; DD, sodium sulfate; EE, nutrient solution having four times the ordinary concentration (cf. table 6).

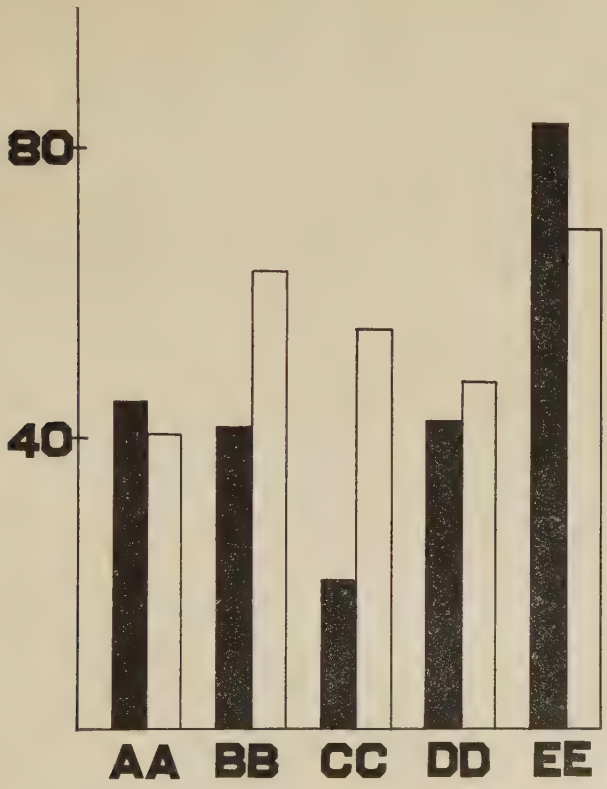

Diagram 1. Representation of the comparative growth of walnut seedlings from cultures in which different sodium salts were present in equivalent amounts. Shaded columns represent fresh weight of tops; unshaded columns, fresh weight of roots. AA, sodium nitrate; BB, sodium chlorid; CC, sodium bicarbonate; DD, sodium sulfate; EE, controls. Vertical scale represents green weight in grams.

The growth of the epicotyls may indicate the comparative toxicity of the various solutions, since practically no epicotyls had developed when the seedlings were placed in the cultures. Their development was most retarded in the $NaHCO_3$ series. In the $NaNO_3$ and the NaCl series, the margins of some of the leaves died showing what may be called salt burn. In the $NaHCO_3$ series the leaves on the epicotyls were small, and in many cases the epicotyls did not emerge from the seed.

The primary roots (initially 8 to 15 cm. long) made small growth in all the Na series. The laterals produced later were short and swollen at the tips like those shown in other experiments with toxic salts. When the experiment was ended the roots in the toxic solutions were brown, though still living. The plants in the control series had well developed leaves and healthy white roots. Some idea of the toxicity of 1000 p.p.m. Na may be obtained by comparing the growth of seedlings in the $NaNO_3$ series with that in the control series. In the former there was a NO_3 concentration of 2692 p.p.m., while in the latter the NO_3 concentration was 2872 p.p.m. In another experiment a concentration of 2692 p.p.m. NO_3 added to the same nutrient solution in the form of $Ca(NO_3)_2$ gave as good growth as the control solution.

The analyses recorded in table 6 show that the Na content of the tops and roots was high and rather uniform when the seedlings had grown in the $NaNO_3$, NaCl, or Na_2SO_4 series. Those grown in $NaHCO_3$ had more Na in their tops than the other plants, but had practically the same percentage in the roots. It will be noted that the tops which made the least growth had the most Na in their ash. The ash of plants from the control series contained approximately the same percentage of Na as one usually finds in similar plants which have grown in less concentrated nutrient solutions.

The K content of the tops was reasonably uniform, though there was some reduction in the K content of the roots.

The effects of $NaNO_3$, NaCl, and Na_2SO_4 were not widely different, but $NaHCO_3$ was more toxic than the other three salts used. Both the tops and roots of the seedlings showed qualitative differences which were not always evident from the weights of those parts. We have found that seasonal conditions may influence the results.

An earlier experiment in which nontoxic concentrations of salts were employed may be cited.[20] Rough-lemon seedlings were grown in nutrient solution containing equivalent concentrations of calcium

in conjunction with NO_3, SO_4, Cl, and CO_3. In the concentrations employed, the growth of roots was influenced more by the amount of calcium in solution than by the character of the anion with which the calcium was combined.

The effects of anions upon the growth of oat seedlings reported by Plate[15] afford an interesting comparison, although he used dilute acids rather than salts, and root growth was inhibited in all the solutions. The size of the oat shoot at the end of the fourteenth day in the different solutions was as follows: $PO_4 > SO_4 > NO_3 > Cl$.

VI. The Effect of Alkalinity on Growth and Sap Reaction

a. Walnut seedlings.

The object of this experiment was to test the effect of a $Ca(OH)_2$ solution maintained at P_H 9.0 upon the reaction of the sap of roots and tops. Two jars of 40-liter capacity were filled with tap water to which $Ca(OH)_2$ solution was added. Twelve walnut seedlings were placed in each jar through perforated covers so that their roots were immersed in the solution. They grew from April 13 to May 9. The P_H of the solution was maintained at or very near 9.0 by allowing a slow stream of saturated $Ca(OH)_2$ solution to flow continually into the culture solution.[21] The P_H of the culture solution was tested at least three times a day and the flow of the $Ca(OH)_2$ solution was regulated accordingly.

During the 26 days of this experiment the plants showed no injury, the roots were well developed though the tops seemed spindling. The fresh weights of tops and roots of 10 seedlings were 53 and 92 grams, respectively. The P_H of the juice expressed from the tops and roots was determined by means of the hydrogen electrode, and was 5.26 and 5.48, respectively. The acid nature of the plant juices agrees with our former determinations and with those of Theron.[25] It seems that the plant contains substances capable of acting as buffers which prevent the change of reaction in the sap.

Although the per cents of Ca found in ash of the tops and roots were 14.64 and 9.80, respectively, nevertheless the P_H of their sap showed no significant changes from the values obtained when such plants are grown in complete culture solutions.

In another experiment walnut seedlings were grown in tap water containing enough $Ca(OH)_2$ to raise the P_H considerably above 9.0. Within a few hours the roots were badly discolored; shortly afterwards

the laterals and the apical portion of the main root died. No new additions of $Ca(OH)_2$ solution were made and four days later the P_H of the solution had fallen to 7.2.

After the extreme alkaline conditions disappeared new laterals were produced not only from the white portions of the primary roots of the seedlings but also from the discolored parts of the primary roots, indicating that in the injured portion at least parts of the vascular system were still alive. The subsequent growth of laterals was very good. The original injury appears to have been confined to the cells of the parenchyma and meristem. Supplementary evidence on this question was afforded by the recovery of roots which had been held for a short time in culture solutions having a slightly injurious reaction. The surface of the roots was discolored. In a short time the reaction was brought to a P_H near 9.0 at which the roots resumed growth.

The subsequent elongation of the central column of root cells broke the discolored layer of cortical cells in the region of greatest growth and produced a banded surface. Similar injury and recovery has been observed when roots had been kept in solutions containing chlorids or other deleterious salts.

b. Wheat seedlings.

Four 40-liter jars were planted with small wheat seedlings. Two culture jars contained a modified nutrient solution maintained as near P_H 6 as possible, the other two jars contained the same modified solution to which enough $Ca(OH)_2$ solution was added to maintain a P_H of 8.0. The composition of the modified nutrient solution expressed as p.p.m. was as follows:

Na	K	Ca	Mg	Fe	Cl	NO_3	SO_4	PO_4	Total
7	496	33	54	1	10	819	216	105	1741

The P_H of the solutions was adjusted three times each day and the solutions were renewed every two or three weeks during the two months of the experiment. Sufficient ferric tartrate was added periodically to prevent the appearance of chlorosis.

After two months the green weight of 20 plants from the culture at P_H 6.0 was 209 g.; the same number from the culture at P_H 8.0 weighed 498 g. It is quite likely that part of the increased growth at the higher P_H was due to increased supply of Ca. The plants from both cultures appeared healthy.

The data in table 7 show that there was no effect on the P_H of tops due to the addition of $Ca(OH)_2$ to the culture solution, but that the P_H of roots was slightly affected. Hurd[7] and Haas[2] have reported an increased P_H in the tops of wheat plants grown in soil to which calcium carbonate was added. On the other hand Newton[14] and the writers found no such increase when plants were grown in water cultures. It would appear that the plants contain substances acting as buffers which maintain a constancy of reaction in spite of differences in the reaction of the medium or of changes in the ion composition of the sap. Similar conclusions were given in another paragraph based upon studies of the sap of walnut seedlings grown at different P_H.

TABLE 7

THE EFFECT OF ADDED $Ca(OH)_2$ UPON WHEAT SEEDLINGS

P_H of culture solution	6.0	8.0
Green weight of tops per 20 plants	173 g.	440 g.
Green weight of roots per 20 plants	36 g.	58 g.
P_H of sap of tops	6.1	6.1
P_H of sap of roots	6.7	7.2
Calcium content of 20 c.c. sap from tops	.0017 g.	.0027 g.

VII. THE RELATION OF H-ION CONCENTRATION TO THE ABSORPTION OF CHLORIDS

a. Citrus seedlings.

The experiments were designed to determine the amount of chlorin absorbed by citrus seedlings from solutions of various H-ion concentration. Rough-lemon seedlings (*Citrus limonia* Osbeck) were grown and all of the plants were from seeds of a single tree. The cultures were grown in enameled-ware pails of ten liters capacity, each of which had approximately 65 seedlings supported on a perforated wooden cover (fig. 8). The solutions were renewed at intervals of three to four days and their reactions were adjusted three times each day.*

The culture solutions was based on that employed by Hoagland. Sodium chlorid was added in amounts equivalent to 1000 p.p.m. (table

* The writers are indebted to A. E. Michelbacher for the careful manipulation of details connected with this work.

8), and hydrates were added to produce the desired concentration of OH-ions. The concentration of sodium chlorid employed is not detrimental to the growth of citrus seedlings in the time involved in these experiments.

TABLE 8

COMPOSITION OF NUTRIENT SOLUTION TO WHICH 1000 p.p.m. NaCl HAD BEEN ADDED

Parts per million										
Na	K	Ca	Mg	Fe	Mn	Cl	NO_3	SO_4	PO_4	Total concentration
400	185	159	54	1	0.1	617	718	216	105	2455.1

Fig. 8. Rough-lemon seedlings grown in water cultures maintained at different hydrogen-ion concentrations. Numbers indicate P_H values maintained. Plants in upper row were taken from cultures in which calcium hydrate was used to maintain P_H values above 6.0; in lower row sodium hydrate was used.

In the first experiment (series FF, table 9), begun on March 14, rough-lemon seedlings were placed in four solutions of P_H 6, 7, 8, and 9. The P_H at which it was possible to maintain the original solution for a few hours with no additions of acid or alkali ranged between 5.5 and 6.0 but usually was close to P_H 6, and we have here designated this solution as P_H 6. The three higher P_H values were maintained by the frequent addition of calcium hydrate. The second experiment (series GG, table 9) was begun March 22, using seedlings from the

TABLE 9

AMOUNTS OF VARIOUS IONS PER 100 ROUGH-LEMON SEEDLINGS GROWN IN HOAGLAND'S SOLUTION +1000 p.p.m. NaCl AT VARIOUS P_H VALUES

Series	FF				GG				Amounts of various ions in 100 rough-lemon seedlings, cotyledons included, taken from germinating box of sphagnum moss.
	$Ca(OH)_2$ used to raise P_H value above 6.0 Seedlings 31 days old				NaOH used to raise P_H value above 6.0 Seedlings 23 days old				
P_H of culture solutions	6.0	7.0	8.0	9.0	6.0	7.0	8.0	9.0	
	g.	g.	g.	g.	g.	g.	g.	g.	g.
Green weight	33.30	61.40	72.30	46.90	28.80	29.40	39.40	31.90	21.80
Dry weight	7.02	9.44	10.67	8.12	5.40	3.99	5.25	5.23	2.53
Ash	.648	1.420	1.367	.952	.502	.532	.680	.580	.197
Na	.072	.135	.147	.090	.042	.058	.062	.065	.020
K	.145	.258	.306	.212	.118	.121	.168	.141	.055
Ca	.041	.165	.105	.090	.032	.045	.048	.032	.011
Mg	.015	.028	.027	.020	.012	.013	.014	.014	.006
Cl	.051	.115	.132	.096	.036	.037	.054	.056	.004
SO_4	.037	.094	.098	.042	.027	.039	.047	.036	.014
PO_4	.095	.211	.164	.075	.084	.089	.068	.056	.054

same lot as those used in the first. Here the three higher P_H values were obtained by the addition of sodium hydrate. On April 14 the seedlings were photographed, harvested and prepared for analysis.

Figure 8 shows the growth in the two series. The former seedlings were grown 31 days and were therefore larger than the latter which were grown 23 days. The seedlings grown in culture solutions maintained at P_H 7, 8, and 9 showed marked improvement over those grown at P_H 6.

The seedlings grown at P_H 6 although they grew slowly, made practically the same type of growth as that simultaneously obtained with unmodified Hoagland's solution. The seedlings put out one or two pairs of new leaves which had a good color and were vigorous in every respect. The greatest growth of tops was obtained at P_H 8 in both sets. The tops of seedlings at P_H 8 were superior to those at P_H 6, but the differences between those at P_H 6, 7, and 9 are not significant. The greatest contrast was in the growth of the root systems. In the $Ca(OH)_2$ series the roots developed richly branching systems at P_H 7 and 8, but at P_H 9 the growth was more limited. In the NaOH series the roots were superior at the higher P_H values to those in the culture at P_H 6.

Table 9 shows the response of the rough-lemon seedlings to the several culture solutions used. The data represent the weight of seedlings and their composition, in grams per 100 seedlings. The initial composition of seedlings is shown in the last column in the table and serves as a means of judging the amount of absorption which occurred.

The weights and photographs show that the plants made good growth in all cases, yet the higher hydroxyl-ion concentration appears to have stimulated growth. In series FF part of the increased growth at P_H 8.0 might have been due to the increased amount of calcium present, but this explanation does not apply to series GG.

The increased growth of seedlings in solutions above P_H 6.0 was generally accompanied by an increase in the amounts of various ions absorbed. It appears that plants grown at P_H 7.0 and 8.0 absorbed slightly greater amounts of sodium than those grown at P_H 6 in both series, while those at P_H 9.0 showed a very small increase. In series FF the plants at P_H 7.0 absorbed twice as much chlorin as those grown at P_H 6.0. At P_H 8.0 there was a still greater absorption, but at P_H 9.0 it was less than at P_H 7.0. In series GG similar amounts of chlorin were absorbed at P_H 6.0 and 7.0, with an increase at P_H 8.0 and 9.0.

The net absorption of sodium and chlorin for the 31-day period is represented by graphs in diagram 2, together with the ash and the green weight per 100 plants. The data show that these seedlings absorbed a larger quantity of both anions and cations from neutral or alkaline solutions than from the slightly acid solution, and made a correspondingly greater growth.

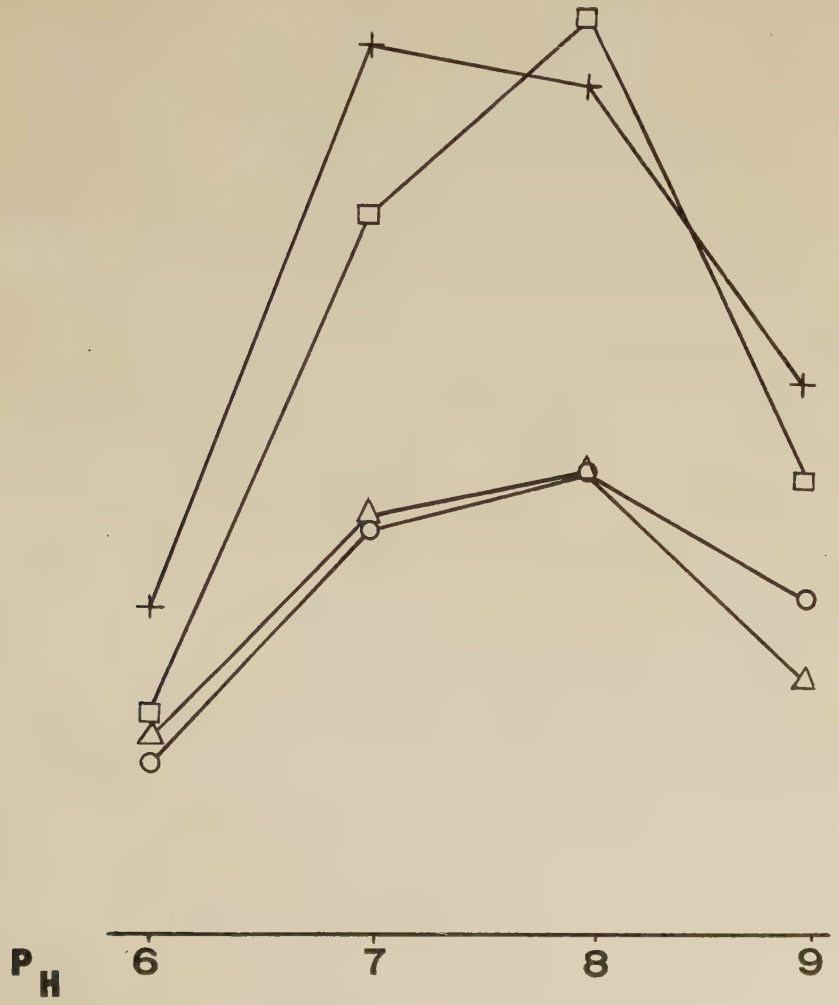

Diagram 2. Growth and absorption of rough-lemon seedlings in nutrient solutions of different P_H values. □, green weight of plants ÷ 200; +, weight of ash ÷ 5; Δ, sodium; O, chlorin.

Table 10 shows the composition of the tops and roots of the same seedlings, expressed as percentages of the ash. The percentage of ash in the tops and roots of both series was greatest when grown in solutions with P_H values of 7.0 and 8.0. It should be noted that in this case, and in some others the percentages were greater at P_H 9.0 than at P_H 6.0.

The percentage of cations in the ash (table 10) shows little evidence for the idea that plants absorb relatively more cations from alkaline solutions. The tops and roots of series FF showed no significant differences in the percentage of sodium at different P_H values. In series GG the plants from the solutions of higher P_H values were in five cases out of six somewhat richer in sodium. It will be remembered, however, that the P_H of these solutions was raised by the addition of NaOH, and that their sodium content was somewhat increased.

With the possible exception of the tops of series GG kept at P_H 8, the percentage of calcium showed a marked increase when the plants were grown in solutions of P_H 7.0. This fact is more noteworthy when we remember that the higher P_H values in series FF were produced by the addition of $Ca(OH)_2$.

The percentage of chlorin in the ash of the tops is smaller in the plants grown in the solution having an acid reaction than in those grown in neutral or alkaline solutions. The smallest percentage of chlorin in both series was found in the roots which grew in the neutral solution, while the percentages in other solutions were quite uniform.

With the exception of the roots in series FF the percentage of sulfate was greatest in plants grown at P_H 7.0 or 8.0. In series GG the percentages of sulfate in the ash of tops and roots were approximately the same at P_H 6.0 and P_H 9.0.

The results presented in this paper open a field in which profitable work may be done with other species of plants and in other ranges of hydrogen and hydroxyl-ion concentrations. It is more than probable that acid or alkaline conditions affect, not only the equilibria between the components of the soil, but the equilibrium between the soil solution and the plant.

Some of Theron's results[25] indicate that the amount of growth and the kind of plants used as well as the ratio of certain ions in the solution may be factors affecting the absorption of the various ions at the different P_H values. The evidence previously published indicates that optimum culture solutions for plants generally have a P_H value of less

TABLE 10

COMPOSITION OF ROUGH-LEMON SEEDLINGS GROWN IN HOAGLAND'S SOLUTION+1000 p.p.m. NaCl AT VARIOUS P_H VALUES

Series		FF				GG			
		$Ca(OH)_2$ used to raise P_H above 6.0 Seedlings 31 days old				NaOH used to raise P_H above 6.0 Seedlings 23 days old			
P_H of culture solutions		6.0	7.0	8.0	9.0	6.0	7.0	8.0	9.0
Tops	Per cent ash in dry matter	9.08	12.56	12.16	11.30	9.06	12.90	12.83	11.37
	Na	12.37	12.73	11.51	11.09	8.85	10.06	8.00	11.78
	K	18.74	17.78	17.99	17.50	19.96	19.89	19.72	18.36
	Ca	7.37	11.41	9.16	9.09	7.65	8.68	9.14	6.26
	Mg	2.34	2.30	1.93	1.72	2.31	2.37	2.25	2.14
	Cl	6.52	8.99	7.88	8.99	5.14	5.55	5.31	8.07
	SO_4	2.68	4.45	3.94	2.52	2.90	3.93	3.16	3.05
	PO_4	12.31	10.93	10.13	8.28	14.78	13.51	11.01	9.49
Roots	Per cent ash in dry matter	9.69	20.90	14.22	12.44	9.93	14.49	13.18	10.91
	Na	7.90	4.11	9.53	6.48	7.47	13.02	11.52	9.46
	K	32.77	19.27	32.00	31.15	31.93	28.97	35.35	35.59
	Ca	3.33	12.55	4.92	9.93	3.58	7.63	2.92	3.74
	Mg	2.13	1.60	2.02	2.53	2.40	2.12	2.00	2.76
	Cl	12.37	7.48	12.20	12.24	12.11	9.90	13.63	12.42
	SO_4	14.07	9.74	13.52	8.00	11.59	14.90	14.90	12.42
	PO_4	21.22	20.75	15.67	7.11	21.40	23.54	8.10	9.23

than 7.0, and that OH ions are more harmful than equivalent concentrations of H ions. It is worth noting that the previous experimental work was largely done with seedlings of cereals or legumes, and it is not surprising to find that plants of another genus may react differently. Within a certain range of P_H values a given concentration of OH ions was less harmful to rough-lemon seedlings than an equivalent concentration of H ions. The graphs in diagram 2 show a certain consistency between green weight, ash, sodium, and chlorin content at different P_H values. These data, together with the appearance of the plants, indicate that conditions for absorption and growth were definitely superior in the solution of P_H 8.0.

b. Walnut seedlings.

Walnut seedling cultures were made in 40-liter jars containing Hoagland's solution to which 1000 p.p.m. NaCl was added. The higher P_H values in one set were maintained by the addition of NaOH, and in the other by $Ca(OH)_2$. The solutions were changed at intervals of 10 to 14 days and the reactions were adjusted thrice daily. Twelve seedlings were put into each solution on March 27 and allowed to grow until May 9. The plants in all cultures made uniformly good growth as shown by the fresh weights of plants (table 11).

The ash of tops and roots of plants from the different solutions had a rather uniform Cl content, although the roots were uniformly richer in Cl than the tops. It would appear from these figures that the H-ion concentration of the media had no appreciable effect either upon the growth of the plants or upon their chlorin absorption.

The sap was pressed from the tops and roots of three plants from each culture. The P_H, as determined by the use of the hydrogen electrode (table 11), shows no significant differences. Hoagland and Davis[6] found a similar constancy of P_H in the sap of Nitella from solutions whose P_H ranged from 5.0 to 9.0.

The analysis of the ash of the walnut seedlings also showed remarkable constancy of composition in most cases. Hoagland[5] reports similarly that there was no general relation between the reaction of the plant sap and the nature of the ion which the plant had absorbed in excess. There is no evidence in these figures for the earlier, and frequently repeated, idea that Na and K are to a certain extent interchangeable in the plant. The percentages of K and Mg showed no significant variations corresponding to the P_H of the culture solutions.

The idea that proteins may combine with anions or cations, according to the hydrogen-ion concentration of the solution, has led some workers to assume that acid solutions bathing plant roots favor anion absorption and that alkaline solutions favor cation absorption. Our

TABLE 11

EFFECT OF P_H OF CULTURE SOLUTION UPON GROWTH AND ABSORPTION BY WALNUT SEEDLINGS

	Series HH							
P_H of culture solution	6.0 to 6.5		7.0		8.0		9.0	
	Tops	Roots	Tops	Roots	Tops	Roots	Tops	Roots
Fresh weight per 10 plants (grams)	93	110						
NaOH series			80	95	95	123	81	107
$Ca(OH)_2$ series			78	121	93	102	70	106
P_H of the sap of the plants	5.31	5.68						
NaOH series			5.48	5.83	5.31	5.82	5.31	5.87
$Ca(OH)_2$ series			5.36	5.75	5.43	5.75	5.26	5.77
Per cent Na in the ash	8.72	17.03						
NaOH series			9.93	20.81	10.64	21.30	10.45	19.96
$Ca(OH)_2$ series			10.22	15.21	8.53	17.13	9.65	16.77
Per cent K in the ash	27.77	21.87						
NaOH series			30.34	19.82	30.61	20.16	33.07	18.77
$Ca(OH)_2$ series			29.80	21.79	29.59	23.81	31.60	21.01
Per cent Ca in the ash	11.18	7.36						
NaOH series			9.76	7.87	9.23	6.42	7.08	7.01
$Ca(OH)_2$ series			10.90	9.05	12.00	7.44	10.27	8.48
Per cent Mg in the ash	5.24	2.44						
NaOH series			5.05	1.62	5.03	2.56	5.01	2.67
$Ca(OH)_2$ series			5.15	2.55	4.90	2.49	4.81	2.60
Per cent Cl in ash of plants	4.98	8.10						
NaOH series			4.87	7.45	4.92	7.78	4.96	8.97
$Ca(OH)_2$ series			4.04	7.20	3.36	8.15	4.00	8.71

results, however, seem to indicate that the neutral point (P_H 7.0) is not a point of great importance so far as absorption by plants is concerned. In other words, there is no evident reason for assuming that the neutral point of distilled water is a sort of turning point, or isoelectric point, in regard to absorption of ions from a solution by plant roots.

VIII. The Effects of Sodium Chlorid on Orange Trees in Soil, and the Results of Leaching

When citrus trees are grown in soils containing harmful amounts of saline material, the leaves are often the first organs to show injury. For a time the leaves appear yellowish, then the margins and apical regions die, and finally an excessive abscission of leaves occurs which makes the unhealthy condition of the trees very conspicuous. Loughridge[12] and Hilgard[3] were among the first to emphasize the toxicity of

TABLE 12

Volume of Liquids Applied to the Cultures, and Transpiration of Orange Trees

(Figures in parenthesis were not included in making averages)

Tree	Volume of liquids added (liters)					Drainage water (liters)	Transpiration (liters)	Ratio of transpiration to dry weight of tree
	Nutrient solution	NaCl solution	Distilled water	Tap water	Total			
84	60	.....	81	274	415	0	415	436
85	(30)	.....	(77)	(146)	(253)	0	(253)	(357)
86	63	.....	93	304	460	0	460	465
87	60	.....	103	328	491	0	491	.....
88	60	.....	104	306	470	0	470	381
89	69	40	33	58	200	46	154	.....
90	69	42	39	173	323	31	292	603
91	66	39	45	212	362	31	331	519
92	66	24	37	204	331	31	300	472

sodium chlorid to citrus trees. More recently Kelley and Thomas[9] have added to our knowledge of the effects of saline irrigation water. The present writers[18] have described the results produced experimentally when young orange trees were grown in sand cultures to which solutions of sodium chlorid were regularly added. We have noted that the chlorophyll of the leaves had a tendency to fade, and that premature abscission was the rule, except where sufficient amounts of calcium salts were present. Twigs and roots were restricted in their growth and eventually many were killed. Rudolfs[24] has reported similar effects of sodium chlorid on other species of trees.

In the following pages we give additional data on the effects experimentally produced by the addition of sodium chlorid to sand and to soil cultures, with especial reference to the chlorid content of orange

leaves, and to the changes which occurred when the soil impregnated with salt was leached with water. The importance of the results lies largely in the fact that we have here produced definite symptoms by the application of a known cause.

A. Trees under controlled conditions.

Young orange (*Citrus sinensis*) trees were grown in large galvanized-iron cans containing sand or soil under conditions which permitted of simple and convenient observation. The details of the general cultural methods have been given elsewhere.[17] The trees were planted May 20, 1920, and removed in February, 1922.

1. *Sodium chlorid added to non-saline soil.*

Trees 13 and 16 were grown in silica sand which received nutrient solution plus 1000 parts per million of sodium chlorid. The other trees were grown in cans which contained soil taken from an uncultivated area on the Citrus Experiment Station property. The soil has been classified as Sierra loam and occurs in regions devoted to citrus culture in the vicinity of Riverside.

Trees 84–88 grew in the soil described above and were irrigated for the first eleven months with distilled water. Trees 89–92 were grown in soil from the same source and were irrigated for the same period with a solution containing 1500 parts per million of sodium chlorid. The latter were seriously injured and showed symptoms which will be described later. During the first eleven months there was no drainage water from the soil. In the latter part of April, 1921, cultures 89–92 were leached with tap water until, as shown in table 12, 30 to 46 liters had been recovered from each container. From that time nutrient solution was added to all cultures to furnish the trees a more nearly optimum supply of nutrient ions.

(*a*) *Changes in the soil.*

When the soil from cultures irrigated for five months with distilled water was sampled on October 8, the amount of material extracted with distilled water (table 13) was about half that obtained five months earlier. The concentration of HCO_3 ions was much diminished and the NO_3 concentration increased from nothing to an appreciable amount. The concentrations of SO_4 and of Cl anions showed no significant change.

The solutes extractable with water eleven months after installing the cultures showed slight differences in concentration from those observed at the expiration of five months. The soil of cans 89–92 gave extracts which contained more Ca, Mg, Na, and Cl than that from cultures receiving distilled water. At the end of eleven months their total content of soluble solids had increased from 589 to 1033 parts per million but the P_H of the extract showed little change.

TABLE 13

ANALYSIS OF SOIL USED IN EXPERIMENTS ON THE EFFECT OF SODIUM CHLORID

(Calculated on the basis of air-dry soil)

	Samples taken when experiment was installed	Cultures 84–88, which received distilled water		Cultures 89–92, which received 1500 p.p.m. sodium chlorid	
	May 20, 1920	Oct. 8, 1920	Apr. 14, 1921	Oct. 8, 1920	Apr. 14, 1921
	p.p.m.	p.p.m.	p.p.m.	p.p.m.	p.p.m.
SiO_2	.....	27	26	25	42
Ca	.....	28	24	53	91
Mg	.....	9	9	16	28
Na	.....	13	22	107	191
HCO_3	183	64	101	61	115
CO_3	0	.....	.....	.....	.....
Cl	18	24	28	221	436
NO_3	0	66	11	58	48
SO_4	22	19	10	15	33
PO_4	18	.....	.....	.....	.....
Total solids as sulphates	400	178	194	589	1033
P_H	.....	6.7	6.8	6.7	7.0

The soil in cultures 89–92 was leached with distilled water on April 15, 1921, until percolates began to appear. Only the first portion of the percolate was alkaline to phenolphthalein, although subsequent portions were alkaline to other indicators. A filtered sample of the first 500 c.c. of percolate from culture 92 showed 12 parts per million CO_3 and 67 parts per million of HCO_3. On the following day the percolates obtained from cans 89 and 92 were still alkaline to phenolphthalein. The application of additional quantities of distilled water followed by nutrient solution finally gave percolates which had P_H values below 8.3.

The first portions of the percolates from cans 87 and 88 showed no alkalinity to phenolphthalein. The results of this and subsequent determinations are shown in table 14. The third column of table 14 contains the P_H values of the percolates obtained at the conclusion of the leaching process which had been in progress for three weeks.

The concentration of chlorin in the percolate varied considerably from time to time. The chlorin content of the percolate from can 92 was small, more or less in keeping with the relative amount of sodium chlorid added. The point of interest was the relatively extensive and the long continued formation of black alkali in the soil of this container in comparison with the soils of cans 90 and 91, which received considerably larger amounts of the salt.

TABLE 14

DATA ON THE PERCOLATES FROM VARIOUS CULTURES

Culture	Grams of NaCl added	P_H of percolates after continued leaching 21 days	Parts per million of chlorid in percolates		
			After 1 day	After 6 days	After 15 days
87	0.0	.....	25	.....	.....
88	0.0	.....	21	.....	.....
89	59.3	7.8	780	984	1179
90	63.4	6.8	255	1167	701
91	58.8	7.0	280	1186	423
92	35.5	7.8	248	44	199

Nutrient solution was added from time to time during the leaching process as well as afterward, and both distilled and tap water were used to supply the soil moisture after the salt treatment was discontinued.

(b) Effects on the trees.

The trees in cultures 84–88, which received only distilled water, grew fairly well for some time, but eventually showed the symptoms commonly observed when the supply of nitrates is insufficient. After a lapse of several months, the soils were irrigated from time to time with Hoagland's nutrient solution. The effect of the nutrient solution was shown in the improved appearance of the trees and in their increased growth. When the trees were removed from the cultures their condition was good and the weight of the various parts (table 15) exceeded that of trees grown simultaneously in sand receiving continuous applications of nutrient solution.

The trees in cultures 89–92 grew only a short time before they began to show the harmful effects of the sodium chlorid in the irrigation water. The leaves eventually became yellow and showed dead margins and tips which are characteristic of salt injury (fig. 9). These leaves fell after a time and were followed by a new crop which usually showed more actute symptoms of injury. The symptoms known as "mottle-leaf" did not appear on any of the trees receiving saline irrigation water. We may note in passing that the percolate from these

TABLE 15

DATA ON THE TREES GROWN IN SOIL CULTURES

(Figures in parenthesis not included in the averages)

Tree	Number of leaves on tree at conclusion of experiment	Dry weight (grams)					
		Leaves	Shoots	Trunk	Root	Rootlets	Total
84	995	224	132	171	197	227	951
85	(1042)	(206)	(96)	(141)	(135)	(130)	(708)
86	1172	311	126	234	184	234	989
87	(873)	.....	(148)	(293)	(256)	(239)	.....
88	730	194	152	368	316	204	1234
Average	966	210	137	258	232	222	1058
89	(473)	.....	(36)	(159)	(103)	(39)	.....
90	464	117	75	123	94	75	484
91	707	153	95	139	136	115	638
92	623	139	89	170	136	101	635
Average	598	136	86	144	122	97	586

cultures at the time when the injury was very severe contained 91 parts per million of calcium (table 13), which may have been responsible for the absence of mottle-leaf on these trees, although it was not sufficient to prevent tip burn. Cummins and Kelley[1] have reported that sodium salts, when applied to soil, set free calcium, and further applications of sodium salts brought about an excessive concentration of soluble sodium in the soil solution, and eventually the soil became deflocculated and impervious.

The depauperate leaves shown in fig. 9 are quite representative of the effects of saline irrigation water on these trees. None of them attained the size usually reached by leaves of the Valencia orange tree. The tissue on one side of the midrib frequently made less growth than

that on the other, with the resulting formation of asymmetrical leaves. In February, 1921 (two months prior to making the photograph), the leaves of trees 90 and 91 showed a considerable amount of tip burn and the older leaves were falling rapidly. Trees 89–92 produced a large number of flowers, in this respect resembling unhealthy trees in the field. The bark on the trunks of trees 89–92 was killed in areas near the surface of the soil. The dead areas were above the surface of the soil and could not have been due to contact with the saline

Fig. 9. Orange leaves from tree which was injured by applications of 1500 p.p.m. sodium chlorid.

irrigation water, since special care was used to prevent it from coming in contact with the trunks. The trunks of trees 84–88 were free from this type of injury. The shoot and root growth of trees 89–92 was notably limited.

In April, 1921, the soil of cans 89 to 92 was leached with distilled water. Leaching of the soil and removal of the drainage water were carried on while the trees were *in situ,* hence any ill effects due to saturation of the soil during the leaching process should have been evident. The leaching process greatly benefited the trees, as will be shown subsequently.

Shortly after the first drainage water was obtained, finely divided colloidal material appeared in it and the rate of percolation was

greatly reduced. Hoagland's nutrient solution was then added in small amounts from time to time, care being taken not to have an excess of solution standing for any long time on the surface of the soil. Frequent additions of solution and removals of drainage water were made, the method followed being in imitation of the removal of salts from precipitates on a filter.

The leaching process naturally removed salts which would have been useful to the trees, and the addition of nutrient solution was therefore advisable if the series were to be at all comparable with that which received no sodium chlorid. The total volume of nutrient solution applied to the soils in series 89–92 was somewhat in excess of that given to the other series (table 12), nevertheless it did not produce in the ten months during which no sodium chlorid was

TABLE 16

P_H AND OSMOTIC PRESSURE OF THE SAP OF MATURE LEAVES

Series	P_H	Osmotic pressure (average of 2 determinations)
84–88	5.80	21.03
89–92	5.82	20.32

applied, trees equal to those produced in the other series. The dry weight of the trees was only about half that of the trees grown in the soil to which no sodium chlorid was added.

Determinations of the hydrogen-ion concentration and of the osmotic pressure of the sap of mature leaves were made May 26, 1921. The former was electrometrically determined and the latter was computed from the lowering of the freezing point. Table 16 shows no significant differences in the P_H of the leaf sap, though as we have seen, the roots of the trees were growing in a soil which had received large concentrations of saline irrigation water and which upon being leached had shown black alkali in the drainage water. Rudolfs[24] has expressed the belief that chlorin increases the acidity in the plant cell, accelerating or harming the vital activities according to the amount employed, but the above results indicate that the chlorin had no appreciable effect on the P_H of the plant sap. A large salt content in leaves may tend to keep a given P_H more constant rather than to change the P_H value.

The trees in both series made very satisfactory growth during the summer of 1921. The appearance of the trees on August 25 may be seen in fig. 11, which shows material improvement over the condition four months earlier (fig. 10). The new foliage produced in series 89–92 was free from the unfavorable characters shown previously.

Fig. 10. Trees in the experiment described. From left to right; trees 87, 88, 89, 90, and 91. Trees 87 and 88 grew eleven months in soil which received distilled water. The other trees grew in similar soil and received water containing 1500 parts per million of sodium chlorid.

Fig. 11. Trees in the experiment at the end of sixteen months. From left to right; trees 88, 89, 90, 91, and 92. The four trees on the right show recovery from the injury induced by the application of sodium chlorid to the soil, and resemble the typical control tree 88 (on the left).

The difference between the volume of water (or solution) added and that of the percolate has been reckoned as transpiration (table 12). While there may be some error in this procedure, it is common to all the trees. The amount of water lost by direct evaporation from the cans was small in comparison with that transpired by the foliage of

the trees. The ratio of transpiration to dry weight is somewhat greater in the case of the less thrifty trees in series 89–92. Data in table 16 show that the osmotic pressure of the sap of the leaves of the control series was not significantly greater than that of the sap of the injured trees at the time when leaching began.

(*c*) *The chlorin content of the leaves.*

Broadly speaking we may say that the addition of sodium chlorid to the soil produced no significant changes in the composition of the ash of the leaves except in respect to their sodium and chlorin content (table 17). Without further evidence we are not justified in assuming that there was a substitution of sodium for potassium.

TABLE 17

THE EFFECT OF SODIUM CHLORID UPON THE COMPOSITION OF ORANGE LEAVES
(Expressed as a percentage of the ash)

	Sand cultures	Soil cultures		
	Trees 13–16	Trees 84–88	Trees 89–92	
	p.p.m.	p.p.m.	p.p.m.	p.p.m.
Na in culture solution	400	0	590	590
Cl in culture solution	898	0	910	910
Condition of leaves	No injury.	No injury.	Injured.	Injured, about to fall.
Constituents of the ash:				
Na	1.61	1.64	4.38	3.98
K	38.25	8.82	9.86	8.85
Ca	8.77	29.77	28.55	26.85
Mg	1.15	2.36	2.78	2.65
Cl	4.73	0.28	17.03	21.36
SO_4	.....	2.36	2.27	2.55
PO_4	.....	3.71	5.26	3.88
Ash expressed as a per cent of dry matter	16.82	14.78	17.54	17.19

The ratio of soluble to insoluble constituents in the leaves was determined by extracting the dry-ground-leaf material with an excess of distilled water for several hours, filtering, and analyzing the soluble and insoluble portions separately. The ratio between the soluble and insoluble portions of each constituent is given in table 18. In the

case of the individual ions there is a significant difference in Na and PO_4 and a puzzling difference in the case of SO_4. The others show no more variation than we are accustomed to find in this kind of work. The proportion of soluble PO_4 in the dried material bears a sort of inverse relationship to the total amounts present, although further evidence is necessary before we can attach much significance to these values.

TABLE 18

THE RELATIVE SOLUBILITY OF THE ASH CONSTITUENTS OF VALENCIA ORANGE LEAVES GROWN IN SOIL. FIGURES REPRESENT PERCENTAGE OF THE TOTAL AMOUNT FOUND.

Source of leaves	Trees 84–88	Trees 89–92	
Condition of leaves	No injury	Injured	Injured, about to fall
	Soluble	Soluble	Soluble
Ash	67.51	70.80	71.15
Na	67.82	74.92	90.97
K	94.48	93.12	97.84
Ca	61.23	62.38	61.85
Mg	92.76	93.17	94.58
Cl	100.00	100.00	99.87
SO_4	75.86	81.44	62.23
PO_4	73.90	54.90	60.53

The percentage of soluble Cl is of great importance in view of the greatly increased amounts of this element which the injured leaves contained. The fact that all the Cl in the dried leaf material was soluble, gives some idea of the cause of the increasing deleterious effects which are associated with the absorption of chlorids. There is no evidence that the chlorin ions migrate from the leaves before abscission.

2. *Soil previously made saline by orchard irrigation.*

Valencia orange trees were also planted in cultures like those previously described to test the possibility of removing excessive amounts of salts under controlled conditions. The soil* was obtained from an orange grove which had been injured by saline irrigation water of the following composition:

Ca	Mg	Na	Cl	CO_3	HCO_3	SO_4	NO_3	SiO_2	*Total solids as sulphates*
172	58	228	627	0	213	76	28	39	1578

* The soil on which the grove stands has been classified by the Bureau of Soils as Ramona loam.

When the soil samples were obtained from the grove we noted that practically all the functioning roots were found between 12 and 24 inches from the surface. Samples of the first 12 inches and of the second 12 inches were collected, each lot being kept separate. Sixteen large galvanized-iron cans were filled with the soil: five cans with the first foot layer, five with the second foot layer, and six with a mixture of equal parts of the two layers. A Valencia orange tree was planted in each can May 21, 1920. Three of the cans in each

TABLE 19

COMPOSITION OF THE WATER EXTRACTS OF SALINE SOIL USED IN CULTURES FOUR MONTHS AFTER THE EXPERIMENT WAS BEGUN, EXPRESSED AS PARTS PER MILLION OF AIR-DRY SOIL

	First foot		Second foot		Mixture of first and second foot	
	Unleached	Leached	Unleached	Leached	Unleached	Leached
Ca	325	95	15	8	132	50
Mg	100	34	5	2	42	20
Na	438	247	207	87	340	247
Cl	1002	390	200	89	566	334
CO_3	0	0	0	0	0	0
HCO_3	107	122	115	137	82	112
SO_4	227	97	52	37	122	90
NO_3	361	156	47	11	221	130
SiO_2	46	48	56	45	45	45
Total solids as sulphates	2950	1251	714	420	1710	1030
P_H	7.0	7.0	7.0	6.8	6.9	7.0
Tap water added (liters)	12.8	40.0	14.3	40.0	13.0	40.0
Drainage recovered (liters)	0	23.9	0	13.0	0	15.3

set were leached and the others were kept as near the optimum water content as possible. The amounts of soluble salt in the soil and the effect of leaching are shown by data in table 19 The total quantities of water and solution applied to the leached soils were about four times those given to the unleached soils. Analyses of the percolates showed a rather constant fall in chlorid content during the first year. Because of the effects of the leaching process upon the soil, we therefore applied a culture solution from time to time.

In a general way the growth of the trees in the several cultures reflected the content of soluble matter in the soils. The trees in the unleached first-foot soil were short lived. They sent out shoots which

TABLE 20

VOLUME OF LIQUIDS ADDED AND GROWTH OF ORANGE TREES IN CULTURES IN SALINE SOIL

	Water applied	Nutrient solution applied	Average number of leaves on tree	Average dry weight per tree (grams)					
				Leaves	Shoots	Trunks	Roots	Rootlets	Total
	Liters	*Liters*							
First foot, leached [1]	268	57	704	123	77	145	127	110	582
Second foot, unleached	132	0	279	26	35	116	79	28	284
Second foot, leached	419	63	845	127	91	218	161	193	790
Mixture of first and second foot, unleached [1]	124	0	326	48	30	106	85	37	306
Mixture of first and second foot, leached [2]	360	60	754	143	96	128	123	195	685

[1] Two of the three trees in the set died and new trees were installed four months after the experiment was begun.

[2] One of the three trees in the set failed to start growth and was replaced by a new tree four months after the experiment was begun.

reached a length of one or two inches, but failed to develop further. The leaves died before reaching their usual size; a little later the new shoots also died. No recovery occurred, in spite of careful attention. The trees in the other unleached soil made restricted growth, and showed marked evidence of salt injury quite similar to that observed where sodium chlorid was added. The leaves were small, yellowish, and dead at the tips. No tendency to mottle-leaf was observed in any of these trees. Table 20 gives the average dry weights of the

Fig. 12. Trees grown in leached and unleached saline soil. Tree in center grown in unleached second-foot soil from an orange grove; other two trees grown in leached second-foot soil.

trees at the end of the experiment, and represents the relative growth made by the several series. The best growth was produced in the second-foot soil. Figure 12 shows certain trees in the leached and unleached soils just prior to the termination of the experiment in December, 1922. The amounts of chlorin in the ash of certain trees are given in table 21, and supplement the data given in table 17.

TABLE 21

PERCENTAGE OF CHLORIN IN THE ASH OF ORANGE TREES GROWN IN UNLEACHED SALINE SOIL

	Leaves	Shoots	Trunks	Roots	Rootlets
First foot, unleached	.....	.....	4.45	11.99	.....
Second foot, unleached	11.79	2.71	1.98	3.28	3.58

B. Trees grown in the field.

Samples of leaves and shoots were collected in April from mature orange trees which had been severely injured by the application of saline irrigation water. For purposes of comparison, samples of leaves and shoots were also taken from healthy trees of the same age in an adjoining grove which had received non-saline irrigation water. The water applied to the injured trees contained 480 p.p.m chlorin

Fig. 13. Young shoots from orange trees in the field showing severe injury following the use of saline irrigation water.

and 200 of sodium. Samples of the soil from this grove were collected at the time the leaves and shoots were taken from the trees. Analyses of the soil samples made by S. M. Brown showed the following amounts of chlorin: 1st foot, 229 p.p.m.; 2nd foot, 211 p.p.m.; 3d foot, 90 p.p.m.; and 4th foot, 99 p.p.m.

The shoots from the injured trees were of two kinds, young and old. There was a region below the tip of each young shoot (fig. 13) which was discolored and shrunken. The older shoots were more or less completely defoliated and were putting out weak, unhealthy secondary shoots, many of which fell after making very feeble growth.

The young leaves were often greatly reduced in size and showed discolored, blistered surfaces (fig. 14). Leaves which reached the usual size often had dead tips or margins. The old leaves which were collected in April had been produced the preceding year and were dead at the tips and margins (fig. 15). At the time they were collected, many of them were falling from the trees. Table 22 shows the

Fig. 14. Young leaves from shoots like those shown in figure 13. The surfaces of these leaves had a blistered appearance.

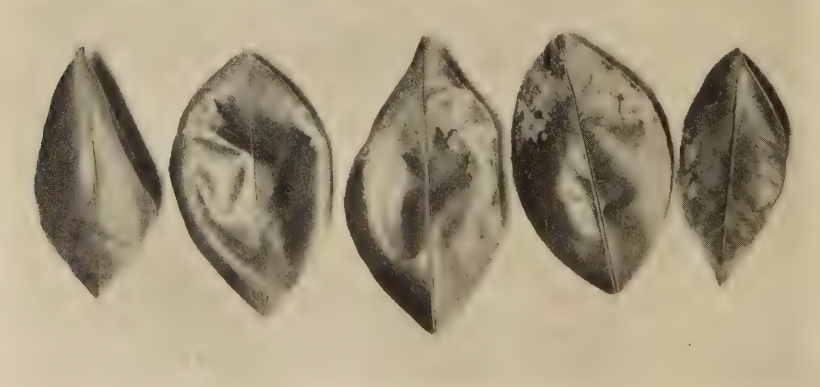

Fig. 15. Old leaves from orange trees which showed severe injury from saline irrigation water.

composition of the shoots and leaves. The very high percentage of chlorin in the shoots and leaves of the injured trees is one of the conspicuous features of this table. The percentage of chlorin in the young shoots and leaves was greater than that in the older organs. It may be well that only the organs which contained the smaller amounts of chlorin were able to survive. Another interesting feature of the analyses is the small percentage of sodium in the old injured leaves.

TABLE 22

THE COMPOSITION OF SHOOTS AND LEAVES OF ORANGE TREES IRRIGATED WITH SALINE AND NON-SALINE WATER

	Irrigated with saline water					Irrigated with non-saline water	
	Shoots		Leaves			Shoots	Leaves
	Young succulent shoots of the last cycle	Defoliated woody shoots of the preceding cycle	Young leaves showing injury	Old leaves still attached but injured	Old leaves fallen as a result of injury	Woody shoots of the preceding cycle	Old leaves not injured
Constituents of the ash:							
Na	5.78	2.12	4.71	1.28	1.19	1.19	2.23
K	18.45	5.85	19.41	3.55	2.75	6.92	5.22
Ca	18.91	29.00	17.05	30.80	32.10	31.30	32.00
Mg	4.52	4.38	3.96	4.02	4.29	2.62	2.23
Cl	22.64	7.23	22.70	15.57	14.45	0.14	0.33
SO_4	1.98	4.46	2.40	3.58	3.73	2.43	3.11
PO_4	6.04	5.81	7.83	2.18	1.48	4.48	1.99
Ash expressed as per cent of the dry matter	10.59	8.51	10.28	17.64	18.03	4.93	12.65

The percentage of ash in the dry matter of the woody shoots and of the old leaves on the injured trees was considerably greater than that of organs of the same age from healthy trees.

The results of these experiments and analyses extend our knowledge of the effects of sodium chlorid on orange trees. They agree in showing reduced growth of roots, shoots, and leaves. Eventually the trees suffer from premature defoliation. The analyses show that the Na and Cl content of injured shoots is greater than that of healthy shoots.

It is also shown that harmful amounts of sodium chlorid may be leached from a soil and that satisfactory growth conditions may be produced if suitable nutrient salts are added soon after the leaching.

IX. The Effect of Sodium Bicarbonate on Young Orange Trees

In the present studies, Valencia orange trees (*Citrus sinensis* Osbeck), were grown in sand cultures to which modified Hoagland's solution containing 1000 p.p.m sodium bicarbonate was added. The technique of the care of the cultures was the same as that previously described. Table 23 shows the concentration of the various ions in the culture solutions and the salts employed. The solution applied to trees 30–35 contained no calcium, while that of the other two series contained 159 p.p.m. The P_H of the solution applied to trees 30–35 was 7.55, while that of trees 38, 40 and 41 was 7.45. The trees were planted in the containers of sand on May 21, 1920, and were removed on September 20, 1921, having grown during the same period as trees 6–11, 12 and 16, 18–23, and 27 and 28, the data on which have already been reported in previous publications.[17, 18]

TABLE 23

Composition of Culture Solutions Employed in Experiments on the Effects of Bicarbonates on Orange Trees

(Parts per million)

	Trees		
	1 and 2	30–35	38, 40, 41
Na	7	280	280
K	185	496	496
Mn	0.1	0.1	0.1
Ca	159	0	159
Mg	54	54	54
Fe	1	1	1
Cl	10	10	291
NO_3	718	718	718
SO_4	216	214	214
HCO_3		725	725
PO_4	105	105	105
Total concentration	1455.1	2603.1	3043.1
Salts employed in making the nutrient solution.	KNO_3 $MgSO_4+7H_2O$ NaCl $Ca(NO_3)_2+4H_2O$ KH_2PO_4 $MnSO_4$ Ferric tartrate	KNO_3 $MgSO_4+7H_2O$ NaCl KNO_3 KH_2PO_4 $NaHCO_3$ $MnSO_4$ Ferric tartrate	KNO_3 $MgSO_4+7H_2O$ NaCl KNO_3 KH_2PO_4 $NaHCO_3$ $CaCl_2+2H_2O$ $MnSO_4$ Ferric tartrate

TABLE 24

NUMBER OF LEAVES, DRY WEIGHT OF VARIOUS PORTIONS OF CITRUS TREES AND WATER REQUIREMENT

Tree	Number of leaves	Dry weight (dried at 60°–65° C.) in grams						Total culture solution added	Total distilled water added	Total drainage water	Transpiration	Water requirement
		Leaves	Shoots	Trunk	Root	Rootlets	Total					
								Liters	*Liters*	*Liters*	*Liters*	
30	52	4.5	7	58	47	9.5	126	184	49	145.5	87.5	690
31	190	27	9.5	134	164	13	347.5	192	61	141.25	111.75	320
32	81	8.5	14	92	103	8	225.5	196	47	138.35	104.65	460
33	218	22	12.5	70	87	17	208.5	186	55	142.80	98.2	470
34	93	19	13	51	55	13.5	151.5	178	41	136.60	82.4	540
35	96	15	24	127	133	13	312	186	56	131.1	110.9	360
Average per tree (30–35)	122	16	13.3	88.7	98.2	12.3	228.5					
38	385	54	26	86	84	39.7	289.7	208	93	109.9	191.1	660
40	330	53	19.5	86	76	34	268.5	208	75	150.75	132.25	490
41	425	84.5	33	80	145	31	373.5	236	75	125.08	185.92	500
Average per tree (38, 40, 41)	380	63.8	26.2	84	101.7	34.9	310.6					

TABLE 25

EFFECTS OF SODIUM SALTS ON ORANGE TREES

Number of leaves, dry weight, and transpiration of average trees of each series

Series	Concentration of ions in addition to that of the control	Number of leaves on average tree	Dry weight (dried at 60°–65° C.) in grams						Transpiration (liters)
			Leaves	Shoots	Trunk	Root	Rootlets	Total	
42–46	Ca of Hoagland's solution replaced by K	110	19	20.8	85.1	115	18.9	258.3	85.9
6–11	393 p.p.m. Na 607 p.p.m. Cl	97	15.6	12.8	91.7	78.9	12.1	211.2	74
18–23	323 p.p.m. Na 674 p.p.m. SO_4	53	14.7	14.6	75.2	107.3	21.8	233.5	95.5
30–35	273 p.p.m. Na 725 p.p.m. HCO_3	122	16	13.3	88.7	98.2	12.3	228.5	99.2
13 and 16	393 p.p.m. Na 159 p.p.m. Ca 888 p.p.m. Cl	498	95.2	35.2	104.2	106	46	386.6	126
27 and 28	323 p.p.m. Na 674 p.p.m. SO_4 159 p.p.m. Ca 281 p.p.m. Cl	749	173	86.5	139	132.5	124	655	183.1
38, 40 and 41	273 p.p.m. Na 725 p.p.m. HCO_3 159 p.p.m. Ca 281 p.p.m. Cl	380	63.8	26.2	84	101.7	34.9	310.6	169.8
1	Control	996	237.1	122.5	194.0	212	136	901.6	228

The data in table 24 show the number of leaves, dry weight, and transpiration of the trees in the two series. Table 25 gives a wider comparison of the composition of the solutions and the average number of leaves, dry weight and transpiration of average trees in these and other series to which sodium salts were added at the rate of 1000 p.p.m. It shows that the growth made by trees 30 -35 was similar to that made by the other trees receiving no calcium. The trees grown with the

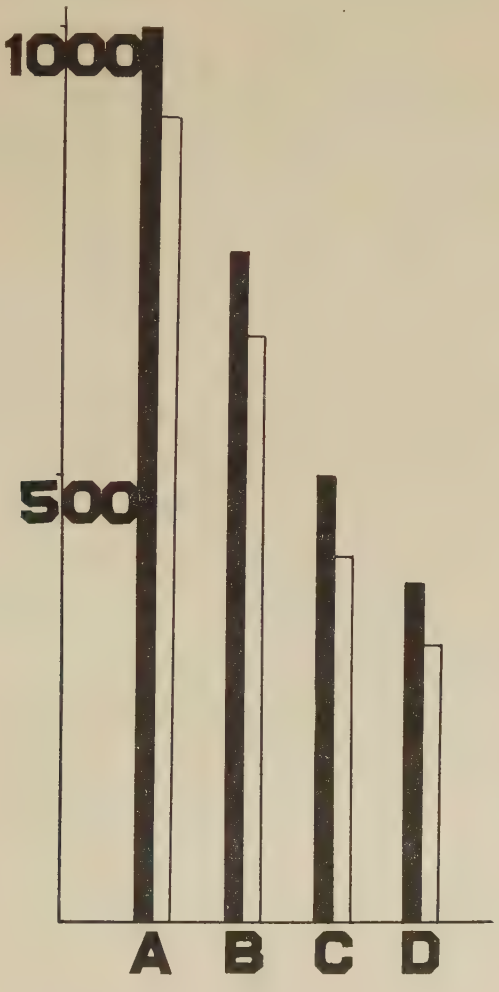

Diagram 3. Comparative effects of three sodium salts upon orange trees. Each salt was added to the culture solution at the rate of 1000 p.p.m. The shaded bars represent number of leaves on the trees, and the unshaded bars represent grams of dry weight of trees. A, complete nutrient solution; B, nutrient solution plus 1000 p.p.m. sodium sulfate; C, nutrient solution plus 1000 p.p.m. sodium chlorid; D, nutrient solution plus 1000 p.p.m. sodium bicarbonate.

additional sulfate (trees 27 and 28) grew better than trees which received the additional chlorin (trees 13 and 16) and these in turn grew better than trees which received bicarbonate (trees 38, 40 and 41). The comparative effects of three sodium salts upon the growth of orange trees are shown in diagram 3.

The effect of bicarbonate on walnut seedlings (table 6) was also more toxic than that of chlorid or sulfate in conjunction with sodium cations.

Fig. 16. Trees 34 and 35 at the end of fifteen months in sand cultures which had received modified nutrient solution lacking calcium and containing 1000 p.p.m. sodium bicarbonate.

Figure 16 shows the tops of trees 34 and 35 on August 25, 1921, after 15 months in cultures which received sodium bicarbonate but no calcium. Many of the leaves had fallen and most of those produced subsequently fell before attaining full size. Many of the shoots became leafless and died. Figure 16 also shows the recurved condition, typical of leaves grown in the absence of calcium (cf. 18, pl. 3, fig. 1). The trunks were still alive when the trees were removed from the containers. The trunk and root system are the last parts of an orange tree to die, under these conditions, due possibly to their retention of calcium and to the slower rate of metabolism in their tissues.

Fig. 17. Trees 38, 40 and 41 at the end of fifteen months in sand cultures which received complete nutrient solution plus 1000 p.p.m. sodium bicarbonate.

Fig. 18. Trees in sand cultures both of which received 1000 p.p.m. sodium bicarbonate. The tree on the left received no calcium, while the tree on the right received 159 p.p.m. calcium as calcium chlorid. The photograph was taken fifteen months after the experiment began.

Figure 17 shows the growth made by the tree tops when sodium bicarbonate was present along with calcium chlorid. The leaves were retained much longer than in the case where calcium was absent from the culture solution. Figure 18 contrasts the condition of the tree tops when calcium is present, with the defoliation that occurs when it is absent. The chlorotic leaves shown in figure 19 are typical of many which appeared on trees 30–35 after a year had passed. These leaves were similar to those which are frequently found on trees in groves where the soil contains bicarbonates and carbonates (Lipman,[11]).

Fig. 19. Chlorotic leaves from trees which received sodium bicarbonate without calcium. Leaves like these never recovered. Scale of inches at the bottom of the picture.

When a condition of chlorosis like that shown by these leaves is attained, no recovery occurs, even though iron is added to the cultures at frequent intervals. The leaves of trees 38, 40 and 41, which received calcium chlorid in addition to sodium bicarbonate, showed no such chlorotic symptoms. The chlorotic condition shown in figure 19 is much more pronounced than that due to the absence of potassium as noted in one of our previous papers[19] (pl. 5, fig. 2), where recovery subsequently followed.

Figure 20 shows the root systems of trees 33 and 35 and of tree 1 which received Hoagland's nutrient solution. Figure 21 shows the root systems of trees 40 and 41 and of tree 1. The root systems of trees 30–35 were very poor, and contained many dead slimy rootlets.

Fig. 20. The effect of solutions containing sodium bicarbonate without calcium on the development of the root system of orange trees. No. 1 grew in a culture receiving a complete nutrient solution; Nos. 33 and 35 grew in cultures receiving nutrient solutions containing 1000 p.p.m. sodium bicarbonate but without calcium (compare fig. 21).

Fig. 21. The effect of solutions containing sodium bicarbonate plus calcium on the development of orange tree roots. No. 1 grew in a culture receiving a complete nutrient solution; Nos. 40 and 41 grew in cultures receiving a complete nutrient solution plus 1000 p.p.m. sodium bicarbonate (compare fig. 20).

The root systems of trees 38, 40 and 41 were small compared with those grown with Hoagland's complete solution, but contained few rotting or slimy rootlets.

Several determinations were made of the P_H of the drainage waters from cans 30–35 and 36–41. It was found to range from that of the culture solution applied to faint alkalinity to phenolphthalein, that is, from P_H 7.5 to P_H 8.5. This indicates that bicarbonates in sand cultures may be converted to some extent into carbonates. The alkalinity present in these sand cultures no doubt greatly reduced the amount of iron available for the rootlets as described in another paper,[22] and also greatly reduced the available supply of phosphate and calcium. The first 500 c.c. of percolate obtained from the cultures upon adding distilled water showed no appreciable change in nitrate from the initial concentration. The phosphate found in the drainage water of series 30–35 showed no change in concentration from that of the solution applied, while that found in the drainage water of series 36–41 showed a reduction to the low average value of 4 p.p.m. Some of the original culture solution for series 36–41 was left standing a few hours and the concentration of phosphate was reduced from 105 p.p.m. to 28 p.p.m.

We cannot ignore the fact, therefore, that one of the potent effects of high OH-ion concentration is to reduce the solubility of certain other ions in the culture solution. In studying the effects of an alkaline solution upon plants, we have to take into account not alone the effect of the hydroxyl-ion concentration upon the plant, but also its effect upon the solubility of the ions in the medium which bathes the rootlets.

Samples of the various portions of trees 30–35 and trees 38, 40 and 41 were analyzed and the results are given in table 26 as percentages of dry matter and of ash.

Total nitrogen and phosphorus were greatest in the poorest trees (30–35). The total sulfur was determined only in the leaves, and by comparison with the SO_4 found in the ash it appears that an appreciable percentage of the total sulfur exists in the organic form. We find that the phosphorus can be largely accounted for as PO_4 in the leaves and shoots, organic combinations of phosphorus being inappreciable in amount.

An important point in the data of table 26 is the very low percentage of sodium in the ash of the leaves and shoots, and the fact

TABLE 26

EFFECTS OF BICARBONATE UPON THE COMPOSITION OF DIFFERENT PARTS OF VALENCIA ORANGE TREES

	(Expressed as per cent of dry matter)										(Expressed as per cent of ash)									
	Leaves		Shoots		Trunk		Roots		Rootlets *		Leaves		Shoots		Trunk		Roots		Rootlets *	
	Trees		Trees		Trees		Trees		Trees		Trees		Trees		Trees		Trees		Trees	
	30–35	38, 40, 41	30–35	38, 40, 41	30–35	38, 40, 41	30–35	38, 40, 41	30–35	38, 40, 41	30–35	38, 40, 41	30–35	38, 40, 41	30–35	38, 40, 41	30–35	38, 40, 41	30–35	38, 40, 41
Total N	2.58	2.36	1.68	1.16	0.79	0.66	0.83	0.69	2.28	1.79										
Total S	0.13	0.23																		
Total P	0.20	0.15	0.18	0.09	0.06	0.04	0.06	0.05	0.24	0.12										
Ash	16.23	19.20	9.58	8.81	4.08	3.61	2.83	3.02	8.62	11.29										
Na	0.12	0.13	0.06	0.07	0.24	0.12	0.31	0.20	0.46	0.56	0.75	0.63	0.57	0.71	5.74	3.15	10.89	6.54	5.30	4.93
K	7.88	8.17	4.04	2.92	0.80	0.79	0.39	0.52	2.45	2.71	48.50	42.55	42.12	33.03	19.69	21.95	13.55	17.10	28.51	23.98
Cl	0.05	0.14	0.02	0.13	0.01	0.05	0.31	0.31	0.33	0.90	0.29	0.70	0.19	1.42	0.24	.145	10.88	10.27	3.87	8.01
Ca	0.33	1.18	0.41	1.06	0.64	0.61	0.42	0.55	0.54	1.39	1.99	6.14	4.28	11.92	15 68	16.76	14.60	18.22	6.34	12.27
Mg	0.30	0.31	0.27	0.31	0.10	0.08	0.09	0.05	0.41	0.39	1.80	1.61	2.80	3.48	2.36	2.02	3.26	1.65	4.70	3.44
SO_4											2.28	2.28	2.23	1.88	1.63	1.77	2.17	1.91	8.44	5.83
PO_4											3.70	2.28	5.86	2.67	3.71	2.76	6.22	4.24	7.49	3.64

* Rootlets calculated to silica-free basis.

that the trunk, root and rootlets contain greater percentages of sodium than the leaves and shoots. Thus far in our studies upon absorption and distribution of elements throughout citrus trees, we have always found a lower percentage of sodium in the ash of leaves and shoots than in the other portions of the tree, and the percentage of sodium has thus far never been found to be relatively high.

All parts of the trees are relatively rich in potassium, the percentage decreasing as we pass from the leaves to the rootlets where there is an increase over that found in the root. The cells of orange trees absorb large amounts of potassium when it is available. In this respect they resemble animal cells, which may accumulate large amounts of potassium, as has been shown by Mitchell and Wilson[13] and others. The amounts of chlorin in the two sets of trees correspond in a way to the concentrations of chlorid furnished them. The repeated losses of older leaves may account for the low Cl content of the leaves, especially in trees 30–35.

The absence of calcium from the culture solution of series 30–35 is reflected most conspicuously in the leaves, shoots, and rootlets, indicating that the calcium of the trunk and root is relatively immobile. Lee[10] has reported the occurrence of mottle-leaf on citrus in the Philippine Islands which is correlated with the species of rootstock employed. It is desirable that studies be made of the calcium requirement and the calcium-transporting power of these rootstocks. At present we cannot say whether a deficiency of calcium in the leaves is due to low calcium mobility or low conductivity through the woody portions of the tree, or whether the alkaline medium which bathes the rootlets has changed the permeability of the cells or has brought about the almost complete precipitation of certain essential ions from the nutrient solution, thereby preventing the plant from obtaining an adequate supply of such ions.

In addition to chlorosis in series 30–35 there was also evidence of mottling. As has been stated, however, the leaves on trees 30–35 fell at so early a stage that it was not possible to follow their condition for any considerable length of time. No initial stages of mottle-leaf were evident in series 36–41, though growth was greatly reduced, the frequent renewal of solution, no doubt, having supplied the trees with sufficient calcium and other ions to maintain themselves. We doubt very much if mottle-leaf is the direct result of an alkaline nutrient

medium. If mottling is found to be present where the culture medium is alkaline, it is possible that it is the result of secondary causes induced by high alkalinity.

X. Relations Between Calcium Deficiency and Chlorosis

In the course of these experiments on the effects of certain salts on the physiology of the orange tree, we have accumulated evidence of a peculiarily close relationship between calcium deficiency and chlorosis. The relation of iron deficiency to chlorosis is well known and it has been shown that a concentration of OH ions which greatly reduces the solubility of iron will induce chlorosis in plants. Several other causes of chlorosis are known so that it is evident that more than one cause may produce this effect. Our object is to call attention to the way in which a deficiency of calcium may cause chlorosis.

We have previously described[18] the chlorotic condition of orange leaves where the trees received a culture solution lacking calcium, but containing sodium salts in considerable quantities. It was shown that such trees lost their leaves prematurely and produced a succession of small chlorotic leaves which were likewise prematurely lost. It is not to be concluded from these observations that the absence of calcium from the culture solution was the sole cause of the chlorosis.

Since the appearance of the publication just cited we have obtained additional data from trees 60–67 grown in sand cultures. The trees were planted in May, 1921, and received a culture solution containing high NaCl and low $CaCl_2$ in the ratio of 98 NaCl: 2 $CaCl_2$. The composition of the solution was the same as that applied to trees 6–11 in the publication just cited, except that it contained the small amount of $CaCl_2$.

For a time trees 60–67 produced good foliage and shoots, but during the winter period of comparative dormancy all the leaves were shed from the trees and many of the shoots died. From the time this symptom appeared the trees were given a complete nutrient solution containing double the usual quantity of salts but lacking the excess NaCl formerly included. New leaves were produced the following spring, but none survived long except those on short shoots arising from the lower portion of the trunk. Many of these leaves were shed and were followed by other crops of leaves. Most of the leaves on the short shoots reached full size, but very few had the normal green color.

Some of them were entirely albescent, in some the veins were green though the tissue between them was pale green or yellow (fig. 22).

When the trees were removed at the termination of the experiment, we found that the greater part of the root system was dead. It appeared that the trees had first produced a fair root system, but that they reached a limit when the small amount of Ca at their disposal was inadequate. The terminal parts of many of the older roots died and then new roots were produced from the living region near the primary root, but many of the second roots likewise died back for

Fig. 22. Chlorotic leaves from orange trees in cultures which received a small amount of calcium in comparison with sodium.

a distance from the apical end. The failure of the trees to produce new roots after the addition of a complete nutrient solution seems to be related to the absence of green foliage. The lack of a suitable supply of carbohydrates may have been an important factor in producing this condition.

We have found chlorotic trees in cultures where the calcium supply was not limited but where faulty drainage caused the roots to die. In such cases the leaves were not shed, and the trees recovered after suitable drainage was provided. The chlorotic condition of leaves generally appears when orange roots die and decay to any considerable extent.

We found that the addition of sodium bicarbonate to the culture solufion restricted the growth of roots and shoots and caused a definite

type of chlorosis where calcium was deficient. It is more than likely that the alkalinity of the culture solutions tended to precipitate certain ions, though the injury observed was not due entirely to the deficiency of nutrient ions.

XI. Interrelations Between Potassium and Calcium

There is an increasing amount of evidence of an opposite relationship between the proportions of potassium and calcium in the citrus tree. Kelley and Cummins[8] have reported on the relationships between these ions in healthy and mottled leaves of certain species of citrus growing in the field. They showed that citrus leaves affected with "mottle-leaf" contain more K and less Ca than normal leaves of the same age. The writers[18] have published further evidence obtained from young orange trees grown in sand cultures under controlled conditions. The ash of trees grown in control cultures contained approximately 25 per cent K and 19 per cent Ca. The ash of trees to which calcium-deficient solutions were applied contained approximately 50 per cent K and 1 per cent Ca. Conversely, the ash of trees to which potassium-deficient solutions were applied contained approximately 30 per cent Ca and 1 per cent K. The writers have found other instances which indicate that the relations between K and Ca in the citrus tree stand in intimate connection with growth and other physiological processes.

Four series of trees in sand cultures (table 27) were grown under conditions previously described. The control series JJ (trees 105-110) received Hoagland's nutrient solution, double strength; series KK (trees 42–46) received nutrient solution (single strength) in which K was substituted for Ca; series LL (trees 111–116) received nutrient solution containing 311 p.p.m. additional K as KCl; series MM (trees 123–129) was like the series KK with the addition of 15 p.p.m. Ca as $Ca(NO_3)_2$.

The trees in the control series JJ made very satisfactory growth and showed no indications of mottle-leaf at any time during the course of the experiment. The leaves were dark green; the shoots and roots were well developed. The trees in series KK made very restricted growth. Their leaves showed the effects of calcium deficiency previously described, viz., premature abscission, chlorosis accompanied by small spots of dead tissue, and abnormal curling. Their roots grew

TABLE 27

GROWTH AND COMPOSITION OF YOUNG VALENCIA ORANGE TREES IN RELATION TO POTASSIUM AND CALCIUM

Series	JJ				KK				LL				MM			
Trees	105–110				42–46				111–116				123–129			
Culture solution contained	370 p.p.m. K 318 p.p.m. Ca 108 p.p.m. Mg 20 p.p.m. Cl				496 p.p.m. K 0 p.p.m. Ca 54 p.p.m. Mg 10 p.p.m. Cl				496 p.p.m. K 159 p.p.m. Ca 54 p.p.m. Mg 291 p.p.m. Cl				496 p.p.m. K 15 p.p.m. Ca 54 p.p.m. Mg 10 p.p.m. Cl			
Osmotic pressure of culture solution (atmospheres)	1.452				.708				1.242				.724			
Average number of leaves per tree	1,351				110				1,084				580			
Average dry weight per tree (grams)	1,417				258				1,080				1,078			
Average units of water transpired per unit of dry matter	319				332				343				356			
	K	Ca	Mg	Cl	K	Ca	Mg	Cl	K	Ca	Mg	Cl	K	Ca	Mg	Cl
Per cent in the ash:																
Leaves	18.23	20.88	1.85	.30	50.04	1.28	1.16	.10	30.06	13.15	1.26	3.39	41.38	6.18	1.80	.33
Shoots	14.89	22.95	2.40	.30	44.86	2.98	1.79	Trace.	24.87	16.64	1.93	4.42	39.36	5.58	3.24	.28
Trunks	13.97	22.70	1.68	.39	26.65	16.50	2.81	Trace.	20.34	19.12	1.46	2.32	25.14	15.38	2.81	.25
Roots	15.59	17.73	2.35	.38	24.11	13.49	3.34	Trace.	21.68	13.74	2.15	2.05	23.29	11.39	3.45	.21
Rootlets (silica free basis)	28.33	7.80	3.71	1.57	36.71	4.55	5.95	1.55	33.91	5.19	2.67	9.44	35.48	2.60	4.48	1.25

poorly and died prematurely. These conditions are reflected in the data (table 27) on the number of leaves and dry weight of trees.

The trees in series LL made good growth and were quite similar to those in the control series. Their foliage was different from that of the controls in that many of the young leaves had small yellow areas in the marginal tissue between the larger veins (fig. 23). The remainder of the leaf tissue was dark green. The yellow areas were covered subsequently with small brown papillae which were quite

Fig. 23. Orange leaves from series LL which received nutrient solution plus potassium chlorid. The marginal chlorotic spots show numerous brown papillae.

characteristic of this type of injury. As time went on the yellow areas became pale green, but they never attained the normal green color of the rest of the leaf. These affected leaves had no tendency to premature abscission. The roots of these trees compared very favorably in size and development with those of the control trees.

Trees in series MM made fairly good growth for a time, but later their leaves began to show yellow circular spots in the tissue between veins (fig. 24). These spots soon became confluent and involved much of the leaf area. Most of the leaves affected in this way fell from the trees prematurely and were followed by other crops of leaves which were smaller, were chlorotic about the margins, and bore brown

papillae on the chlorotic area (fig. 25). While the symptoms resembled those noted for leaves in series LL, they were generally indicative of more severe injury. We noted also that the injury was more severe on leaves produced during the hot summer months. Many of the depauperate leaves produced in the succeeding crops had no green tissue except near the veins and midrib (fig. 26) and therefore resemble closely the condition known as "mottle-leaf" or "frenching." The photomicrograph of a leaf section (fig. 27) shows something of the

Fig. 26. Orange leaves from series MM showing the mottled depauperate condition finally attained.

nature of the papillae on the affected leaves. The illustration shows two elevations covered with a thick dense layer. The elevations appear to have been produced by a proliferation of cells of the palisade layers. The thick superficial layer was seldom ruptured and we found no indication of parasites in the affected areas. Only a portion of the leaves on the trees in series MM were affected in the manner described; the remainder made very satisfactory growth without showing any of the symptoms noted.

Fig. 28 shows a representative tree from each of the two series LL and MM. It will be seen that there was more defoliation in the case of MM where only 15 p.p.m. calcium was supplied to the culture than in the case of LL where 159 p.p.m. calcium was supplied.

Fig. 24. Orange leaves from series MM which received nutrient solution deficient in calcium plus potassium chlorid. These leaves show an early stage of injury which resembles mottling.

Fig. 25. Orange leaves from series MM showing more advanced stage of injury. The yellow areas are covered with small brown papillae.

Fig. 29. Orange leaves from trees which received a magnesium-free nutrient solution for two years.

Fig. 27. Photomicrograph of a section of an orange leaf bearing papillae like those shown in figures 23 and 25.

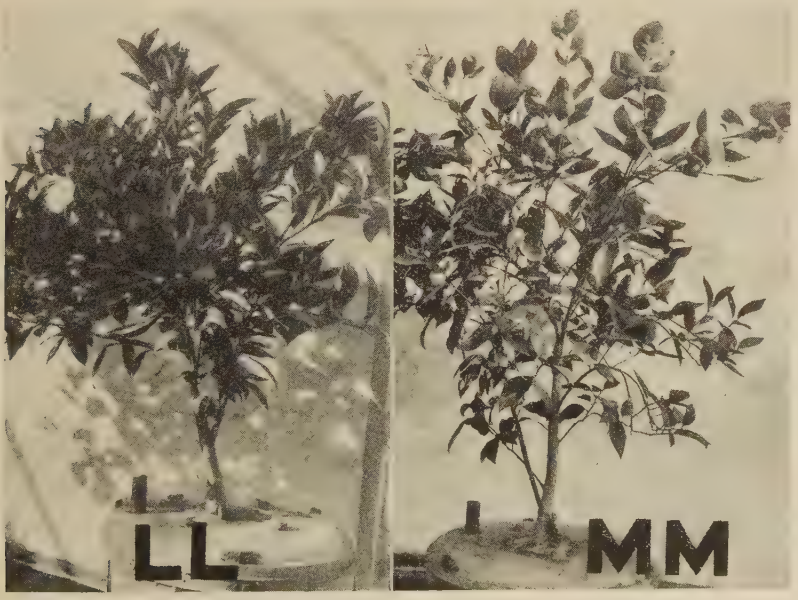

Fig. 28. Representative trees from series LL and MM. The partial defoliation of the trees from series MM is apparent.

The root system of trees in series JJ and LL was large and well developed, but in MM the roots made restricted growth and contained many rotten rootlets.

The percentage of K (table 27) was generally higher in the ash of leaves and rootlets (except when roots died) than in other parts of the trees. The ash of trunks and roots had considerably smaller percentages of K than other parts of the trees. The leaves and shoots of the trees in series KK were notably high in their K content, although the trees received the same concentration of K in the culture solution as trees in series LL and MM. The K content of these trees shows an inverse relationship to Ca content similar to that which has been discussed previously. The parts of the trees which showed the most serious effects were those which had the highest per cent of K in their ash.

The Ca content of the trees corresponded in a qualitative way with the Ca content of the culture solutions, that is, the trees in series KK which received no Ca contained the least calcium, and trees in series JJ which received a solution containing 318 p.p.m. Ca contained most. The trunks and roots in most cases contained more Ca than other parts of the trees.

The variations in the per cent of Mg in the ash were small and bore no apparent relation to the condition of the trees.

The Cl content of the ash was, in no case, high enough to be responsible for any of the injurious effects observed. In fact, the trees in series KK and MM which showed injury contained negligible amounts of Cl.

Analyses of the affected leaves from series MM showed minor differerences in the content of certain ash constituents. Their composition was:

	Per cent of ash	Na	K	Ca	Mg	Cl	SO_4	PO_4
Affected	13.43	9.77	45.27	4.00	1.66	.32	3.86	3.54
Healthy	13.58	9.30	41.38	6.18	1.80	.33	3.79	3.35

The amounts of the ions are expressed as percentages of the ash. The leaves were selected as closely as possible for uniform age. The differences in composition, while small, agree with those found by Kelley and Cummins[8] in the case of normal and mottled leaves.

TABLE 28

GROWTH AND COMPOSITION OF VALENCIA ORANGE TREES IN RELATION TO MAGNESIUM

Series	NN						OO					
Culture solutions contained	14 p.p.m. Na 108 p.p.m. Mg 210 p.p.m. PO_4						110 p.p.m. Na 0 p.p.m. Mg 105 p.p.m. PO_4					
Osmotic pressure of solution	1.452 atmospheres						.965 atmospheres					
Number of leaves per tree	1,351						1,379					
Dry weight per tree	1,417						1,486					
Units of water transpired per unit of dry matter	319						317					
	Na	K	Ca	Mg	SO_4	PO_4	Na	K	Ca	Mg	SO_4	PO_4
Per cent in the ash:												
Leaves	4.58	18.23	20.88	1.85	4.39	4.70	5.98	20.94	19.57	.57	4.07	3.78
Shoots	3.29	14.89	22.95	2.40	4.86	7.77	4.02	14.70	24.66	.62	2.81	5.02
Trunks	3.42	13.97	22.70	1.68	3.75	6.82	5.16	15.35	22.25	1.15	1.56	6.57
Roots	3.71	15.59	17.73	2.35	2.68	8.15	6.68	12.43	17.22	1.17	1.52	6.50
Rootlets (silica-free basis)	7.09	28.33	7.80	3.71	12.52	12.05	12.19	22.09	6.10	.80	12.29	7.51

The results of this experiment bring out some facts concerning the relations between K and Ca which seem worthy of notice. The marked injury shown by trees in series KK was so similar to what has been observed in other cases that it can be ascribed principally to Ca starvation. The yellow spots on the leaves, the premature abscission, and the gelatinous dead roots were characteristic of Ca starvation. The effects of Ca deficiency were so great as to make it difficult to distinguish any other effects.

In series LL the injurious effects were insignificant or at least transient; on the contrary, the injurious effects noted in series MM were obvious and became more severe as time went on. It would appear that, in the latter case, the amount of Ca furnished the trees was sufficient to prevent the most acute results of Ca starvation though it did not prevent the trees from taking up large amounts of K. In the case of series LL, the larger concentration of Ca furnished, changed the proportion of K and Ca in the ash, and allowed the trees to make satisfactory growth.

Attention may be called to the fact that, in a large series of experiments upon the effects of various salts upon orange trees, the writers have not hitherto obtained results which resembled as closely the condition known as "mottle-leaf" as those here reported. It appears to us that the development of mottle-leaf is related to certain conditions in which the amount of K present greatly exceeds that of Ca. This is not to imply that we think there is a particular proportion between K and Ca which causes this condition; on the contrary, there may be a rather wide range of conditions which produce a similar result. The concentration of monovalent ions may be in excess of that of Ca without producing effects on the trees such as here described. For example, another series which received a culture solution containing concentrations in parts per million as follows, K 185, Na 400, Ca 159, and Cl 617, showed none of the symptoms of mottling found in trees of series LL nor of mottling and abscission found in trees MM.

The H-ion concentration of the sap of healthy mature leaves from series LL and MM showed no significant difference. The former had a P_H of 5.82 and the latter 5.92, as determined electrometrically. Their titration curves were practically identical.

XII. Effects of Magnesium Deficiency on Young Orange Trees

Six trees were planted in the sand cultures April 5, 1921, and removed January 5, 1923. The care of the cultures was like that described in the preceding paragraphs. The composition of the magnesium-free culture solution in parts per million of ions was

Na	K	Ca	Mg	Cl	NO_3	SO_4	PO_4	Fe	Mn
110	185	159	0	10	718	214	105	1	0.1

giving a total concentration of 1502.1 parts per million. The control series NN grown simultaneously received double-strength nutrient solution.

The trees in the magnesium-free series 00 grew well and attained a development at the end of the period mentioned equal in all respects to those in the control series NN (table 28). The only significant difference was a peculiar chlorotic stripe on the older leaves and this character did not appear until the experiment had been in progress about a year. The nature and development of this chlorotic condition are shown in fig. 29. Between the larger veins in the initial stages, there was a series of yellow areas situated a short distance on either side of the midrib of the leaf. As these yellow areas enlarge they coalesced to form a well-defined stripe in the middle of the leaf and gradually covered the veins and midrib. The bleaching was most pronounced at the base of the leaf. In course of time the chlorotic area extended toward the margin of the leaf as shown at the right of fig. 29, and eventually many leaves were entirely chlorotic. We noticed no premature abscission of these chlorotic leaves. Only part of the leaves on any tree showed this condition and they were usually in the lower, interior part of the tree.

The composition of various parts of the trees (table 28) shows few significant differences when compared with that of the control trees. The principal difference was in the Mg content, as one might expect. The lowest content of Mg was found in the ash of leaves, shoots, and rootlets. We have previously shown[23] that the Mg of the trunk and root of an orange tree is somewhat less soluble than that of other parts of the tree, and it is possible that the original content of Mg in these trees had not been entirely reduced by transfer to the more actively growing portions of the tree.

Attention may be called to the significance of this fact in the nutrition of trees. Although the tree when planted contained about 2 per cent of Mg in its ash, this small amount was sufficient to maintain good growth and no untoward effects of any kind were observed during the first year. Somewhat similar results were noted when orange trees were deprived of potassium, but, on the contrary, injury was soon apparent when trees were deprived of calcium.[19] Trees deprived of Mg showed no striking changes in their content of other ions, although there was some reduction in the amount of PO_4 in the ash of the rootlets.

In view of these results we may assume that a relatively small amount of magnesium is adequate for satisfactory growth and chlorophyll production. This assumption is supported by the fact that good growth is obtained when the trees are given a nutrient solution containing Mg in a concentration of 54 parts per million.

SUMMARY

The data presented in the preceding pages give some of the results obtained from several series of experiments with seedlings and young trees. They are designed to give some information on the phenomena of absorption of ions and the consequent effects upon growth. Walnut seedlings absorbed sulphate in increased amounts when the concentration of that ion in the culture solution was increased, although the absorption was by no means parallel to the concentration. Seedlings supplied with a culture solution containing 1200 p.p.m. sulphate made very good growth, but, in higher concentrations growth was restricted and leaves were killed at the margins.

Concentrations of nitrate as high as 1600 p.p.m., furnished as sodium nitrate, had no detrimental effects on the growth of walnut seedlings, and higher concentrations only had a retarding influence upon the growth of tops. An initial concentration of 2700 p.p.m. nitrate produced injury to the margins of walnut leaflets similar to that produced by a concentration of 1500 p.p.m. chlorin as NaCl. A comparison of the growth attained in equi-molecular concentrations of sodium nitrate and sodium sulfate indicated that the latter is the less favorable.

Walnut and orange trees absorb chlorin readily and their growth is characteristically affected by amounts which would be harmless in the case of certain other anions. The roots of these seedlings usually contain more chlorin than the tops, though the leaves of orange trees may contain as high as 22 per cent chlorin in their ash. Between P_H 6.0 and 9.0 the reaction of the culture solution seemed to have little influence upon the amount of chlorin in the ash of walnut seedlings. Rough-lemon seedlings grown in a similar range of P_H values had slightly more chlorin in the ash of tops at P_H 9.0 than at lower values. The smallest percentage of chlorin in the ash of their roots was found in plants grown at P_H 7.0, and the amounts in roots grown at P_H 6.0, 8.0, and 9.0 were not significantly different.

Young orange trees grown in soil cultures were seriously injured by the application of irrigation water containing 880 p.p.m. chlorin (1500 p.p.m. NaCl). The leaves turned yellow and showed dead margins which are typical of salt injury. The chlorin content of the injured leaves was very high, and there was no evidence that chlorin migrates from the leaves prior to abscission, although it was soluble in water.

Soils containing harmful amounts of sodium chlorid were leached under controlled conditions and their salt content was reduced to a point which permitted the trees to make satisfactory growth, although it was necessary to add nutrient salts.

A concentration of 1000 p.p.m. of sodium bicarbonate restricts the growth of walnut roots and often prevents completely the development of the epicotyls. The application to young orange trees of culture solution deficient in calcium and containing sodium bicarbonate, retarded the growth of the tops and eventually killed the roots. The orange leaves first showed abnormal curling and translucent spots, followed by premature abscission. Many of the leaves developed chlorosis.

The injurious effects of high concentrations of potassium ions were not evident until considerable time had passed. Walnut seedlings in culture solutions containing 1700 p.p.m. potassium made good growth for a time, but eventually the foliage turned yellow and the roots ceased to grow. Young orange trees in sand cultures which received solutions containing 500 p.p.m. potassium grew well for the first year, but eventually their leaves developed characteristic yellow spots on which brown papillae appeared. Many of the depauperate

leaves subsequently produced resembled mottled leaves. These untoward symptoms were more severe in a series of cultures where the amount of calcium furnished was very small. The leaves and rootlets showed most severe injury from this cause and their ash was richer in potassium.

The data show that sodium is not severely toxic unless the concentration is fairly high. When a sodium salt was added to a complete nutrient solution the trees were not severely injured except in cases where concentration as such became a factor. The growth of the epicotyl of walnut seedlings was retarded by sodium salts more than the growth of roots; however, the roots were characteristically injured later.

Walnut seedlings made thrifty growth in solutions of calcium chlorid, and very restricted growth in equi-molecular solutions of sodium chlorid. Strong concentrations of chlorin as calcium chlorid were somewhat less toxic to rough-lemon seedlings than equivalent concentrations as sodium chlorid. When calcium was furnished in sub-minimal amounts, the roots of young orange trees died and the foliage was chlorotic. The ash of the trunk and the main root of the orange trees was richer in calcium than other parts of the tree, though in certain cases the older leaves were very rich in calcium. Additional evidence may be gathered from these experiments to support the idea that the absorption of calcium and potassium presents a sort of antithesis in the function of the citrus tree. If the amount of one element is high in the ash, the amount of the other will be low. The roots of walnut seedlings are extremely sensitive and are quickly injured in calcium-free solutions.

Culture solutions whose reaction was on the alkaline side of the neutral point were not detrimental to the growth of rough-lemon seedlings; in fact, they made more growth at P_H 8.0 than at P_H 6.0 or lower. Citrus and walnut seedlings grown at P_H values of 7.0, or higher, contained as much chlorin in the ash of their tops as those grown at lower P_H values. The P_H values of the sap expressed from walnut seedlings were quite uniform regardless of the reaction of the solution in which they were grown.

A concentration of 1500 p.p.m. sodium sulfate caused no apparent injury to walnut seedlings, but at a concentration of 3000 to 6000 p.p.m. sodium sulfate the growth of the seedlings was progressively

retarded. Increasing concentrations of sodium nitrate had somewhat the same effect.

It is very difficult to anticipate the effects of a given concentration of an ion in the culture solution upon the amount of that ion found in the plant, although, in a general way the per cent found in the plant reflects the concentration in the culture solution. The data show, however, that the variations are by no means proportional.

On account of its mobility in the plant we might expect to find chlorin rather uniformly distributed, but we find that it has a tendency to accumulate in the roots of orange trees and in the epicotyls of walnut seedlings.

The effect of a very low concentration of magnesium on orange trees was mainly evident in a peculiar type of chlorosis in which only the tissue along the midrib of the leaf was involved. In spite of a low content of magnesium in the ash of the trees, there was no evidence of any profound physiological disturbance.

The effects of sodium chlorid on trees growing in soils under controlled conditions throw some light upon certain conditions often seen in the field. The restricted growth of roots and shoots, and the premature abscission of leaves agree with the analyses which show an increased chlorin content of those members. The conditions for growth in saline soil were greatly improved as a result of leaching the cultures while the trees were growing in them.

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